MERCURY EVOLUTION IN FRESHWATER FISH FROM RESERVOIRS IN NEWFOUNDLAND, CANADA

KENNETH J. FRENCH

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Mercury Evolution in Freshwater Fish from Reservoirs in Newfoundland, Canada

by

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in partial fulfilment of the requirements for the
degree of Master of Science (Biology)

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Abstract:

Return times of 7-12 years have been recently hypothesized for salmonids collected from recently (post-1980) created reservoirs in NF. These return times have been primarily based on reservoir age though factors such as the flooded riparian zone and acidity have all been postulated to affect return times. It was the goal of this study to improve upon predictions of return times for Newfoundland by expanding the current database of mercury in fish and by incorporating several of these factors.

An investigation into sediment mercury levels in 34 non-impounded headwater lakes across NF indicated that acidity had no effect on the amount of mercury accumulated at the lake bottom. Watershed area to lake area ratio (WA:LA) was found to be significant in explaining the variation observed in sediment mercury levels. This suggested that large watersheds can deposit significant amounts of mercury sorbed to organic material to small lakes that then act as sinks for this metal. This relationship was further supported by the positive correlation between lake colour and WA:LA.

The slope of the relationship between mercury concentration and fork length for salmonids was found to change significantly over time. Reservoir age and conductivity were found to be significant in predicting the slope of the relationship between fish mercury levels and fork length for ouananiche (*Salmo salar*) and brook trout (*Salvelinus fontinalis*). Only the per cent Area Flooded was significant in predicting the slope for arctic charr (*Salvelinus alpinus*). Slope variability between the three control ponds and between pre-impoundment and post-impoundment mercury data precluded determination of return times for either species.

An investigation into mercury time series data for a number of impounded and non-impounded sites indicated that high slopes may be characteristic of non-impounded sites and of old reservoirs. Results suggested that impoundment lowers the slope during the first 4 to 5 years following inundation by increasing mercury levels in smaller fish more so than in larger fish. Slopes appeared to return to pre-impoundment levels approximately 5 to 6 years after inundation. Weighted regression indicated that mercury increase in a selected size interval following impoundment was 8 to 73 times faster than the decline after mercury levels peaked.

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List of Abbreviations and Symbols:

Name	Symbol (units)
Acetic acid anion	$COOH^-$ ($\mu eq\ l^{-1}$)
Acidity	pH
Age Reservoir	AR (years)
Alkalinity	Alkal ($\mu eq\ l^{-1}$)
Aluminum	Al^{-3} ($\mu eq\ l^{-1}$)
Analysis of Variance	ANOVA
Area Flooded	AF (ha)
Below Determination	b/t
Calcium	Ca^{2-} ($\mu eq\ l^{-1}$)
Centimetres	cm
Chloride	Cl^- ($\mu eq\ l^{-1}$)
Chlorophyll a	Chl ($\mu g\ l^{-1}$)
Common Logarithm	$\log_{10}$
Conductivity	Cond ($\mu S\ cm^{-1}$)
degrees Celsius	^0C
Department of Fisheries and Oceans	DFO
Dissolved nitrates	Nitrates ($\mu g\ l^{-1}$)
Dissolved oxygen	DO ($mg\ l^{-1}$)
Dissolved Organic Carbon	DOC
Fork length	FL (cm)
grams	g
Hectares	ha
hydrochloric acid	HCl
Linear Polyethylene	LPE

litres	l
Magnesium	Mg^{+2} (μeq l^{-1})
Mercury	Hg
meters	m
Newfoundland and Labrador Hydro	NFLH
normal	N
Not Determined	n/t
N-1-naphthylethylenediamine dihydrochloride	NNED
Particulate organic carbon	POC (μg)
Particulate organic nitrogen	PON(μg)
Parts per million	ppm
Phenyl Arsine Oxide	PAO
Potassium	K^{+} (μeq l^{-1})
Revolutions per minute	rpm
Secchi depth	Secchi depth (m)
Sodium	Na^{+} (μeq l^{-1})
Standard Deviation	Std. Dev.
Sulfate	SO_4^{2-} (μeq l^{-1})
Total Colorimetric Units	TCU
Total phosphorous	TP (μg l^{-1})
Versus	vs
Water Shed Area to Lake Area Ratio	WA:LA

Chapter 1

Introduction: Mercury in Aquatic Food Webs

1.1. Introduction:

Mercury has attracted more attention in the last 20 years as a contaminant of aquatic food chains than any other trace pollutant. Mercury pollution initially emerged as a serious problem in isolated localities near industrial sources in the mid 1900's. More recently, contamination of recreational fish has been associated with reservoir creation, and since 1980, much scientific research has been oriented towards relating mercury contamination of fish to impoundment. Several empirical models have been developed predicting mercury return times following impoundment, but they are quite variable in their estimates, ranging from five to as much as 30 years.

1.2. Mercury and Its Uses:

Mercury, a Group 2B transition metal, is the only metal that exists as a liquid at room temperature (20^{0}C (degrees Celsius)) (Chang, 1986). Although rarer than gold and platinum (mercury constitutes approximately 8 X 10^{-6} per cent (%) of the earth's crust by mass), its sources are so much more concentrated that the metal can be readily obtained (Chang, 1986). Mercury occurs mainly in the form of its ore, mercury(II) sulfide (HgS), better known as cinnabar. Mercury has been known since 5th Century B.C. (Before Christ) (Farrar and Williams, 1977) and it is important to man in both the chemical and agricultural industries. It is used as a catalyst in the production of vinyl chloride and acetaldehyde - two chemicals used in the production of plastics (D'Itri and D'Itri, 1977), and applications range broadly from cosmetics, medicinal treatments, and dentistry to paints, electrical apparatus, and batteries (Fitzgerald and Clarkson, 1991). Alkyl mercury

has been used as a seed disinfectant and phenyl mercury as a fungicide in the pulp and paper industry (Kjell *et al.*, 1988). In addition to its utility, elemental mercury and many mercury compounds are toxic, volatile, and readily dispersed through the atmosphere (Fitzgerald and Clarkson, 1991).

1.3. Mercury Poisoning and Pollution:

Mercury pollution was initially recognized in Japan where Minamata disease puzzled health authorities in the early 1950's (D'Itri *et al.*, 1978). This disease, which includes difficulties with vision, hearing, speaking, and muscular coordination, derives its name from Minamata Bay, Japan where cases of poisoning began to appear in 1953 (Bunce, 1994). By 1960, 1300 people had been poisoned, 200 of which had died (Bunce, 1994). Since the confirmation that Minamata disease resulted from industrial poisoning of fish, researchers have recognized that methyl mercury contamination of food chains leading to man is a health hazard (Fitzgerald and Clarkson, 1991). Cases of mercury poisoning had been known in the felt hat-making industry since the 1800's (D'Itri and D'Itri, 1977), but it was not until 1971 that the mercury problem was recognized in North America. On May 7, 1971 the American swordfish fishery was closed due to mercury contamination of commercial products (Idyll, 1971).

In Canada, elevated levels of mercury in fish were first detected in 1969 in Lake St. Clair and lakes in Northwest Ontario known to have been polluted with mercury released from industrial sources (Fimreite, 1977). During the summer of 1975, a Japanese team invited by the Canadian National Indian Brotherhood visited Grassy Narrows and

White Dog Indian Reserves in Ontario and found that 37 of 89 people known to consume quantities of fish as part of their diet, were showing signs and symptoms of mercury poisoning (Harada *et al.*, 1978); one newborn had a hair concentration of 30,000 ppm (parts per million), equivalent to a blood concentration of 120 ppm (Clarkson, 1978). Since then methyl mercury concentrations have been reported in freshwater fish above the Canadian limit of 0.5 ppm in other areas of Ontario as well as Northwest Quebec (Verdon *et al.*, 1991), Manitoba (Bodaly and Hecky, 1979; Bodaly *et al.*, 1984), Labrador (Anderson *et al.*, 1995), and Newfoundland (Scruton *et al.*, 1994).

Given the toxicity of mercury, measures have been taken to reduce mercury emissions over the last 20 years. However, global atmospheric elemental mercury concentrations have increased at an annual rate of 1.2 to 1.5% between 1977 and 1990 (Slemr and Langer, 1995). Deposition of methyl mercury itself has been measured at several sites in the Northern Hemisphere and has been found to vary considerably from one region to another (Rudd, 1995). Wet bulk deposition values of 0.39 mg $ha^{-1}yr^{-1}$ have been recorded in North West Ontario (St. Louis *et al.*, 1995). North, East, West, and South Sweden have respectively observed deposits of 0.70, 2.0, 2.9, and 1.9-4.0 mg ha^{-1} yr^{-1} (Munthe and Iverfeldt, 1995). Wisconsin has received 0.88 mg ha^{-1} yr^{-1} of methyl mercury deposition (Fitzgerald, 1995). At the present time, the origin of methyl mercury deposition is not known but may be directly or indirectly related to industrial activity (Rudd, 1995). There is now more mercury in terrestrial and aquatic systems than in previous centuries as shown from the analyses of polar ice cores, lake sediment cores, and peat cores; the global atmospheric burden of mercury is estimated to have increased by a

factor of three since 1900 (Pilgrim, 1996).

1.4. Mercury Methylation:

The chemical species of mercury in the environment are of great ecological significance due to their potential toxicity. Mercury can enter the aquatic environment and the food chain via weathering, atmospheric transport, dissolution, vaporization, and biological processes. Three mercury oxidation states occur in nature: metallic or elemental (Hg^0), ionic-mercurous (Hg_2^{2+}), and mercuric (Hg^{2+}) with elemental and mercuric mercury predominant (Cappon, 1994). Inorganic (Hg_2^{2+} and Hg^{2+}) and elemental (Hg^0) mercury pose little hazard except that they are readily transformed to methyl mercury, the most toxic form and the form most readily assimilated and accumulated by biota. Methyl mercury is 100 times more toxic than inorganic mercury (Friberg and Vostal, 1972) and in general, organometallics are more mobile, more toxic, and more readily bioaccumulated (Weber, 1993) than their inorganic forms. Exposure of humans to methyl mercury causes nerve damage, erethism, and even death (D'Itri and D'Itri, 1977).

The process of methylation is not well understood and there is controversy as to the importance of biotic versus abiotic methylation. Our understanding of internal lake production of methyl mercury remains vague due to the absence of methods to measure natural rates of methylation or demethylation (Rudd, 1995). The production of methyl mercury has been considered primarily a biological process (D'Itri *et al.*, 1971; Bodaly and Hecky, 1979; Compeau and Bartha, 1985; Ramlal *et al.*, 1985) occurring within lakes via bacterial methylation of inorganic mercury. Biologically, sulfate-reducing bacteria are

the most important methylators (Compeau and Bartha, 1985; Gilmour and Henry, 1991). This process occurs primarily in aquatic anaerobic sediments where sulfate is limited and mercury can be used as an alternative electron acceptor. Cobalamin, also known as vitamin B_{12}, is produced during fermentation and is the intermediate metabolite that methylates mercury (D'Itri *et al.*, 1977; Weber, 1993; Zillioux *et al.*, 1993; Bunce, 1994). Methyl cobalamin contains a central cobalt atom making the methyl group act somewhat carb-anion like and attacks Hg^{2-}, transferring the methyl group to the mercury cation (Bunce, 1994). The carbon-mercury bond is weak but is also non-polar, so it is not readily cleaved (Bunce, 1994). Thus, it is a relatively stable chemical species.

The requirements for abiotic methylation are the presence of humic acids as organic matter and metal ions acting as catalysts (Lee *et al.*, 1985; Zillioux *et al.*, 1993). There are a number of mechanisms by which inorganic mercury can be abiotically methylated within an aquatic system. Photochemical methylation is believed to account for as much as a 3% conversion of mercuric acetate per day (Summers and Silver, 1978). Methyl cobalamin, humic matter, and methyl tin (Sn) though arising from biotic processes, can non-enzymatically methylate Hg^{2-} (Weber, 1993). The importance of methyl cobalamin in the methylation process remains obscure. Gilmour and Henry (1991) stated that most vitamin B_{12} producing bacteria do not appear to participate in methylation in sediments, and that methylation must require more than B_{12} production. However, Berman *et al.* (1990) found that a sulfate-reducing bacterium released an analog of methyl cobalamin methylated Hg^{2-} in sediments. Compeau and Bartha (1985) observed that methyl cobalamin methylated Hg^{2-} in sediment slurries in the presence of MoO_4^{2-}

(molybdate ion), which inhibits sulfate reducing bacteria. The importance of methyl cobalamin in environmental methylation is still unclear (Weber, 1993). Methyl tin compounds can abiotically methylate Hg^{2+} with transfer of the methyl anion since Hg is a better methyl acceptor than Sn(II) (Weber, 1993). The universal presence of methyl tin in both freshwater and marine environments suggests that this process may occur readily. Mono-, di-, and trimethyl tin species are all common in natural waters (Byrd and Andreae, 1982; Donard *et al.*, 1986). Humic matter, which includes any mixture of natural, metal-complexing organic compounds present in the aquatic environment or extracted from it, has the potential to methylate Hg^{2+} (Weber, 1993). Nagase *et al.* (1982) determined that a high concentration of humic acid (6000 mg l^{-1}) methylated 3800 μM (micro molar) of Hg^{2+}; Craig and Moreton (1985) observed that sterilized humic matter extracts from estuarine sediments methylated 5000 μM of $HgCl_2$ (mercury chloride) and other Hg^{2+} compounds in water; Lee *et al.* (1985) recorded methylation of 100 μM $Hg(NO_3)_2$ (mercury nitrate) or $HgCl_2$ by fulvic acid. In these cases, the formation of methyl mercury is probably the result of an electrophilic attack on fulvic acid by Hg^{2+}(aq) (Weber, 1993). Berma and Bartha (1986) reported that abiotic mechanisms formed up to 21 parts per billion (ppb) of methyl mercury, while biochemical methylation under similar conditions formed up to 288 ppb. This indicated that biological mediation was more important. However, most research on methylation of Hg^{2+} has been done by microbiologists, and there has been a lack of emphasis on the abiotic process (Weber, 1993). Gilmour and Henry (1991) concluded that the information available on methylation in soils was insufficient to judge the importance of abiotic methylation, particularly by humic

substances. Weber (1993) questioned the importance of biotic methylation and indicated that the sufficient but low sulfate concentration needed to produce methyl mercury in coastal and marine sediments does not explain its ubiquitous occurrence, that the high concentrations of Hg^{2+} added to samples in scientific studies eliminates all but mercury resistant species which do not predominate in the aquatic environment, and that sterilization methods used actually change sediment chemistry. Though the importance of biotic versus abiotic methylation is still controversial, the combination of these processes may explain the ubiquitous occurrence of methyl mercury in aquatic environments.

1.5. Detoxification Mechanisms:

Demethylation has the potential to decrease mercury accumulation in fish and reduce transfer through the food chain. Bacteria capable of demethylating organic mercury have been found (D'Itri *et al.*, 1977; Zillioux *et al.*, 1993). This process is enzyme mediated and occurs aerobically. It consists of a two-enzyme system: a hydrolase that hydrolyses the carbon-mercury bond and a reductase that reduces Hg^{2+} to Hg^{0}, which is then volatized at the lake surface (D'Itri *et al.*, 1978).

The importance of demethylation in aquatic systems remains unclear. However, selenium (Se), a Group 6A element, has been found to have ameliorative effects on mercury poisoning. The most consistent beneficial effect of this nonmetal on methyl mercury poisoning has been a reduction in lethal and neurotoxic effects (D'Itri *et al.*, 1978). Selenium as selenite (Se^{-4}) is most effective in preventing methyl mercury neurotoxicity in experimental animals (Cappon, 1994). Addition of methyl mercury to the

diets of quail produced over 90% mortality within two weeks while addition of sodium selenite to methyl mercury-containing diets produced only 21% mortality (Stoewsand *et al.*, 1974). Daily survival was increased 30% in rats fed a methyl mercury and selenium diet as opposed to a selenium-free diet (Friedman *et al.*, 1978). Moreover, specific human populations consuming large quantities of fish high in mercury (>1.0 ppm) and containing natural levels of selenium, have shown no evidence of methyl mercury poisoning (Tsuguyoshi *et al.*, 1980; Cappon, 1994). The amelioration mechanism of selenium for methyl mercury poisoning is not well known. The natural biological sink for methyl mercury is in its interaction with sulfhydryl groups. The selenohydryl group binds methyl mercury 100 times more tightly than the sulfhydryl group, and it is clear that diets supplemented by selenium or with high natural levels achieve an added degree of protection against methyl mercury poisoning (D'Itri *et al.*, 1978). Information gathered establishing the detoxifying effects of selenium on mercury leads to an interesting question of whether or not increasing natural selenium concentrations in freshwater systems could ameliorate heavy metal pollution problems (Rudd *et al.*, 1980).

1.6. Mercury in Hydroelectric Reservoirs:

Mercury pollution has long been associated with industrial sources but recently a different problem has arisen. Elevated mercury levels have been reported in fish from hydroelectric reservoirs (Bodaly *et al.*, 1984; Hecky *et al.*, 1987; Morrison and Thérien, 1991; Verdon *et al.*, 1991; Scruton *et al.*, 1994; Montgomery *et al.*, 1995). These elevated mercury levels are related to reservoir creation because fish in adjacent

undisturbed lakes do not show such signs of increased mercury burden. This occurs in reservoirs free of industrial discharges and hence is due to mercury present in the terrestrial environment prior to flooding and subsequently released as a result of inundation (Bodaly *et al.*, 1984; Hecky *et al.*, 1987; Morrison and Thérien, 1991; Montgomery *et al.*, 1995). The addition of organic compounds from flooded terrain can stimulate mercury methylation (Fagerström and Jernelöv, 1972; Akagi *et al.*, 1979; Wright and Hamilton, 1982; Gilmour, 1992). Increased bacterial production due to the influx of nutrients and the degradation of flooded terrestrial vegetation, peat, and humus can result in release of this terrestrial store of mercury and probably serves to promote mercury methylation (Bodaly *et al.*, 1984) and accumulation in fish.

1.7. Factors Affecting Mercury Methylation:

1.7.1. Sulfate:

Field studies have shown that metal methylation occurs most rapidly in anoxic sediments, in the presence of active microbial sulfate reduction (Compeau and Bartha, 1985; Gilmour *et al.*, 1987). It has been postulated that increased levels of sulfate in freshwater ecosystems will also result in increased rates of transformation of inorganic mercury to methyl mercury, potentially 'fertilizing' mercury methylation by sulfate reducing bacteria (Gilmour and Henry, 1991). However, Steffan *et al.* (1988) found that the amount of methylation was significantly less for sediment samples with sulfate additions than for those without, and the addition of sulfate to freshwater lakes has generally not been shown to stimulate methylation (Winfrey and Rudd, 1990).

1.7.2. Riparian Zone:

Wetlands, bogs, and peatlands have all been linked to locally increased amounts of mercury. Many wetlands accumulate mercury largely as a result of atmospheric deposition, either directly or through transport from the watershed (Zillioux *et al.*, 1993). Krabbenhoft *et al.* (1995) found substantial increases in total mercury and methyl mercury in ground water discharged through peat. Wetland components of watersheds may be important in Hg cycling because of their proximity with streams, lakes, and reservoirs and their high organic carbon content, for which trace metals have a strong affinity (Krabbenhoft *et al.*, 1995). Peatlands and similar zones are high in humic matter and have a high capacity for binding mercury. As well, mosses such as *Sphagnum*, tend to accumulate and retain more mercury than other plants (Huckabee, 1972; Zillioux *et al.*, 1993), because they do not assimilate minerals or water from soil but derive most constituents, including heavy metals, from the atmosphere (Huckabee, 1972). It is these zones that contribute to the methylation process by releasing mercury species into ground or surface water that eventually end up in lakes, reservoirs, and fish. St. Louis *et al.* (1995) found wetlands to be important sources of methyl mercury to a boreal forest lake ecosystem and reported that yields of methyl mercury were 26-79 times higher from wetland portions of catchments than from purely upland areas. The accumulation in superficial soil layers has saved the aquatic environment from the full effects of mercury deposition; at the same time the accumulation has created a terrestrial store of mercury that is a classic example of a 'chemical time bomb' (Bishop *et al.*, 1995).

The Province of Newfoundland is dominated by extensive areas of organic

deposits having in excess of 2 million hectares of peatlands (Rybak *et al.*, 1989); organics can amount to 25-30% of the land types in many of the major river watersheds (Northland Associates, 1989). The rate of decline of fish mercury levels in Newfoundland reservoirs may influenced by the extent of the riparian zone in the inundated area.

1.7.3. Dissolved Organic Carbon (DOC):

The amount of DOC in the water column has been linked to the amount of mercury methylation. Decreased DOC levels increased net methyl mercury production in the water column possibly due to the removal of competitive binding sites for Hg (Winfrey and Rudd, 1990; Miskimmin *et al.*, 1992). DOC plays a complicated role in the transport and availability of mercury species. Presumably, ligand formation between dissolved Hg and DOC in the water column makes mercury unavailable for methylation by bacteria (Gilmour and Henry, 1991). In contrast to these studies, Watras *et al.* (1995) found that high DOC favoured mercury methylation; Driscoll *et al.* (1995) found that total methyl mercury increased with increasing concentrations of DOC, and stated that the most obvious factor regulating the concentration and availability of both total and methyl mercury in Adirondack lakes is DOC. Bodaly *et al.* (1984) proposed that elevated fish mercury levels following impoundment may be a result of increased amounts of organic matter that stimulate the bacterial methylation of mercury. McMurty *et al.* (1989) found that DOC explained a significant amount of the variation observed in fish from Ontario lakes while Grieb *et al.* (1990) observed that fish mercury levels decreased with increasing DOC content in Michigan seepage lakes. However, no significant relationship was found

between total dissolved Hg and DOC in the water column of several natural and artificial aquatic systems of Northern Quebec (Montgomery *et al.*, 1995) while Zillioux *et al.* (1993) concluded that the highest mercury concentrations in drainage waters from wetlands remote from point sources of mercury tend to associate with high levels of DOC. Watras *et al.* (1995) found that Hg and methyl Hg in the surface waters of Northern Wisconsin lakes were both positively correlated with DOC, with DOC accounting for 82-92% and 64% of the variability in Hg and methyl Hg respectively. Watras *et al.* (1995) suggested that there is a lower potential for evasive efflux under conditions of high DOC. There are extensive areas of organic deposits across Newfoundland (Rybak *et al.*, 1989), and DOC may be important in altering the availability of mercury for methylation and for accumulation in reservoir fish.

1.7.4. Productivity:

Impoundment generally increases primary productivity due to the influx of organic matter and nutrients from the inundated area. In Cat Arm reservoir, Newfoundland, Copeman and Knoechel (1986) found that the observed rates of primary production following inundation exceeded the levels previously observed. Nutrient additions to the Rawson lake Basin in the Experimental Lakes Area of Ontario, Canada, resulted in a immediate increase in sediment methyl mercury production (Wright and Hamilton, 1982). Korthals and Winfrey (1987) found low levels of mercury methylation within the water column of an oligotrophic lake and suggested that the low rate of methyl mercury production in the water column was likely due to the small number of microorganisms and

low nutrient concentrations. This may not be reflected further up the food chain. D'Itri *et al.* (1971) found that fish in an unpolluted oligotrophic lake in Michigan had higher mercury levels than fish in a similarly remote eutrophic lake in the same area.

1.7.5. Acidity:

There has been much controversy as to the effect that acidity has on the methylation process and the subsequent accumulation of methyl mercury in fish. Elevated methyl mercury levels in fish from acidic lakes suggest a link between the process of methyl mercury production and acid deposition (Gilmour and Henry, 1991). Survey data indicated that Hg concentrations in fish of a given species and age are often inversely correlated with lake pH or alkalinity and suggested that acidification resulted in increased gill permeability to methyl mercury (Wiener *et al.*, 1990). Miller and Akagi (1979) found that the amount of methyl mercury doubled in the water column for a decrease in pH of 1 to 2 units. Decreased pH resulted in a loss of porewater Hg (Ramlal *et al.*, 1985) and in a loss of DOC and thus a reduction in the number of binding sites for inorganic mercury (Winfrey and Rudd, 1990). The loss of porewater Hg may reduce methylation because mercury must be in solution before it can be methylated (DeSimone *et al.*, 1973). This loss of binding sites may actually increase the availability of mercury for methylation and may also increase the mercury concentration at the bacterial cell surface by promoting binding directly onto the cells (Winfrey and Rudd, 1990).

Ramlal *et al.* (1985) found that mercury was methylated more slowly in lake sediments at lower pH (~4) than it was at natural pH (5-7). Acidification of sediments

with sulfuric acid significantly decreased mercury methylation activity (Furutani *et al.*, 1984; Ramlal *et al.*,1985) and acidification of sediments to pH 4.5 decreased activity by greater than 65% (Steffan *et al.*, 1988). Results imply that the decreased rates of methylation were due to the increased hydrogen ion concentration since additions of sodium sulfate (Na_2SO_4) did not depress or only slightly depressed, methylation (Steffan *et al.*,1988; Gilmour and Henry, 1991). Authors that have reported increased levels of mercury burden in fish from acidified lakes must therefore look towards other mechanisms such as increased gill permeability (Rodgers and Beamish, 1983; Wiener *et al.*, 1990), or altered partitioning of methyl mercury between sediment and water at low pH (Miller and Akagi, 1979). Winfrey and Rudd (1990) hypothesized that decreases in pH reduced mercury volatization at the lake surface by reducing the number of electron donors through removal of DOC for reduction of Hg^{2+} to Hg^0. Moreover, Xun *et al.* (1987) found that decreased pH in Canadian Shield lakes has resulted in increased methylation in the water column. Acidity has been found to affect methylation and mercury accumulation in fish but it remains unclear what role pH has in the mercury cycle.

1.7.6. Trophic Status:

Mercury return time has been linked to the trophic status of fish species in hydroelectric reservoirs. Mercury can accumulate in fish due to uptake from water and food (Phillips and Buhler, 1978). Mercury becomes biomagnified along the food chain, reaching maximum concentrations in top consumers (Cabana *et al.*, 1994). Potter *et al.* (1975) found that there was a positive correlation between food habit and mercury levels

in stomach and axial muscle of fish. Anderson *et al.* (1995) found that piscivorous fish from the Smallwood Reservoir, Labrador, Canada such as Lake trout (*Salvelinus namaycush*) and Northern pike (*Esox lucius*) continued to demonstrate higher levels of mercury than fish from unimpounded sites 20 years after inundation while most non-piscivores had returned to background levels. Brouard *et al.* (1990) found that in non-piscivorous species, maximum levels of mercury were reached ~5 years after impoundment, but 9 years after flooding mercury levels were still increasing in Northern pike. Cabana *et al.* (1994) found that lake trout from lakes with the longest food chains had the highest mercury levels. Therefore, fish mercury levels in Newfoundland reservoirs may be a function of the food habit and the trophic status of individual species.

1.8. Return Times (*OR* the time required for Hg to return to safe levels):

Several studies on mercury evolution in hydroelectric reservoirs have estimated different return times for mercury levels in fish to return to pre-impoundment levels. Some studies postulate the return time to be on the order of 20-30 years (Verdon *et al.*, 1991), while others estimate return times of 7 to 12 years (Scruton *et al.*, 1994), and estimates as low as 3 to 5 years have been made (Abernathy and Cumbie, 1977). However, return times have not incorporated certain characteristics of reservoirs that have been shown to affect the rate of methylation and subsequent bioaccumulation in fish. Such factors include the soil type and the riparian zone of the inundated area, the amount of dissolved organic carbon (DOC), the productivity of the impoundment, the acidity, and the trophic status of fish species.

1.9. Hypotheses:

Models exist predicting the levels of mercury in reservoir fish populations. Johnston *et al.* (1991) have developed empirical models to predict the mercury levels in fish from the Churchill River project in Manitoba and Futter (1994) has developed a model predicting the influence of the pelagic food-web structure on the probability of mercury contamination in lake trout. Recently, return times of 7 to 12 years have been hypothesized for salmonids in Newfoundland based on a number of recently created (post 1980) reservoirs (Scruton *et al.*, 1994). It is the goal of this study to develop models for Newfoundland that will predict return times for fish mercury levels in hydroelectric reservoirs and improve upon or substantiate existing predictions. These models were developed using an expanded database of fish from reservoirs across the Island; they tried to incorporate certain physical, chemical, and biological characteristics of the reservoirs, features not included by Scruton *et al.* (1994). Models were formulated with two basic hypotheses, the first being that mercury levels will be higher in younger reservoirs. The second hypothesis was that the observed levels of mercury will depend on not only the degree of inundation, but also on other biological, chemical, and physical characteristics of the reservoirs, such as the acidity and conductivity.

1.10. Conclusion:

Mercury contamination of aquatic food chains is a well-known phenomenon in both hemispheres of the world. There are biological and abiotic sources of mercury and methyl mercury to aquatic biota and subsequently, man. Mercury in aquatic food chains

increases with impoundment because inundation releases mercury occurring in flooded soil and vegetation. The resulting elevated concentrations of Hg in fish may be influenced by a number of biotic and abiotic factors as is the time required for fish to return to background levels.

Chapter 2

Influence of Physical and Chemical Characteristics on Sediment Mercury Levels in Thirty-Four Headwater Lakes from NF, Canada

2.1. Abstract: Mercury in aquatic sediments represents a potential source of this trace metal to biota, notably fish. Site specific factors, such as acidity and DOC (dissolved organic carbon) have been shown to affect the mobilization of mercury and methylation of mercury. Methyl mercury is the most toxic form of this metal and the form most readily accumulated by biota. Thirty-four headwater lakes, selected for a range in pH, were sampled for sediment mercury levels as part of an investigation of the impacts of acid rain on insular Newfoundland (NF) lakes. Selected physical and chemical data were also collected on all of the study sites. Acidity was not found to be significantly related to sediment mercury concentrations despite the wide range in pH. Pearson correlation analysis indicated that sediment mercury level was positively correlated with WA:LA (watershed to lake area ratio). WA:LA was also correlated with Secchi depth and colour. Linear regression was used to estimate the relationship between sediment mercury and WA:LA. This indicates that direct input of Hg from the watershed by dissolution and sedimentation is a major factor affecting sediment mercury concentrations.

2.2. Introduction:

Since the confirmation that Minamata disease was the direct result of methyl mercury accumulation in fish, researchers have recognised mercury contamination of food chains as a potential health threat to humans (Fitzgerald and Clarkson, 1991). The problems associated with elevated mercury levels in reservoir fish have been recognised for a number of years (Bodaly and Hecky, 1979; Bodaly *et al.*, 1984; Hecky *et al.*, 1987; Morrison and Thérien, 1991; Montgomery *et al.*, 1995). Mercury can enter the aquatic environment and the food chain via weathering, atmospheric deposition, dissolution, vaporization, and biological processes. Inorganic (Hg_2^{2+} and Hg^{2+}) or elemental (Hg^0) mercury pose little risk except that they are readily transformed to methyl mercury. Methyl mercury is the most toxic form and the form most readily assimilated and accumulated by biota, specifically fish. Methyl mercury is 100 times more toxic than inorganic mercury (Friberg and Vostal, 1972). It is the methylation process that poses a threat to humans since exposure to organomercurials causes nerve damage, erethism, and even death (D'Itri and D'Itri, 1977). Meister *et al.* (1979) reported that the mercury in fish originated in the soil indicating that sediments act as a source of mercury to the aquatic environment. The microbial methylation of Hg in sediments has been regarded as the dominant source for the accumulation of methyl mercury in fish (Lee *et al.*, 1985). Little information is available concerning the influence of site specific factors on sediment mercury levels, although relationships between fish mercury levels and other site specific factors have been investigated (Scott and Armstrong, 1972; MacCrimmon *et al.*, 1983; Wren and MacCrimmon, 1983; McMurty *et al.*, 1989; Johnston *et al.*, 1991; Cabana *et*

al., 1994; Watras *et al.*, 1995).

Acidity is thought to influence Hg availability for methylation and bioaccumulation. Elevated mercury in fish from acidic lakes suggests a link between methyl mercury production and acid deposition (Gilmour and Henry, 1991). Further relationships between methyl mercury production (Furutani *et al.*, 1984; Ramlal *et al.*, 1985; Xun *et al.*, 1987; Steffan *et al.*, 1988), levels in fish (Rodgers and Beamish, 1983; Wiener *et al.*, 1990), and partitioning between sediments and the water column (Miller and Akagi, 1979; Xun *et al.*, 1987) in acidified systems have been reported. Jackson *et al.* (1980) found that acidification of water in softwater lakes retarded the removal of Hg from the water column, with the result that at pH 5.1 Hg had a longer residence time than any other true metal. Wiener *et al.* (1990) found that fish Hg concentrations are often inversely correlated with alkalinity and sulfate additions have been found to depress methylation (Steffan *et al.*, 1988; Winfrey and Rudd, 1990). As well, ions such as Cl^-, associated with acid deposition, are involved in the mass transfer of elemental mercury between the gas and aqueous phase (Pleijel and Munthe, 1995) and suggest a pathway towards increased wet deposition and accumulation in sediments.

Other physical, chemical, and geographical features of aquatic systems are thought to affect the availability of Hg for bioaccumulation. Certain factors can stimulate the microbial or abiotic pathway to methyl mercury production and subsequently alter mercury partitioning between sediment and water. The size of the lake may influence inputs into the system through dry or wet deposition. Differences in Hg loadings to lakes may arise from differences in watershed to lake area ratios and from differences in the

retention of Hg by watersheds (Mierle and Ingram, 1990). Nutrient additions to the aquatic ecosystem can result in increased sediment methyl mercury production (Wright and Hamilton, 1982). Abiotic methylation requires humic acids and certain metals acting as catalysts (Weber, 1993). DOC (dissolved organic carbon), a correlate of humic acids, has been found to favour mercury methylation (Driscoll *et al.*, 1995; Watras *et al.*, 1995) and has been linked to fish mercury levels (McMurty *et al.*, 1989; Grieb *et al.*, 1990).

Mercury in sediments is usually in an insoluble biologically unavailable form. However, the accumulation of mercury in sediment represents a risk to resident recreational fish populations. Sediment bound mercury can be liberated and made biologically available via bacterial or abiotic methylation. Conversion of inorganic mercury to methyl mercury results in its desorption from sediment particles at a relatively fast rate and little or no methyl mercury is found in sediments (Menzer, 1991). Methyl mercury is readily accumulated and assimilated by biota. Site specific factors such as acidity and watershed area may enhance the availability of mercury for accumulation in biota by affecting both the amount of mercury entering the system and the methylation process. The relationship between sediment mercury levels and acidity and other site specific factors for a suite of non-impounded headwater lakes is investigated to determine which of these factors influence sediments mercury levels in natural systems.

2.3. Methods and Materials:

2.3.1. Lake Selection:

Lakes considered for this study were selected from an extensive lake inventory database for insular Newfoundland (NF) which included an island-wide survey of 109 headwater lakes conducted in 1981 (Scruton, 1983) and a follow-up survey of 90 lakes on the south coast of the Island in 1983 (Scruton and Taylor, 1989). The subset of 34 lakes used in this investigation were initially selected to develop a surface sediment calibration equation relating fossil diatom abundances to lake pH for use in paleolimnological reconstruction of the pH history of selected lakes (Scruton and Elner, 1986; Rybak *et al.*, 1989; Scruton *et al.*, 1991). This data subset consequently represented the full range of pH identified for insular Newfoundland lakes (Scruton 1985). All study lakes were located along the south coast and on the northern peninsula (Figure 2.1.) and were higher order lakes. Lake order is defined as the position of the lake in the watershed. It is numbered by headwater extension, with Lake order 1 being the first lake in the watershed. Lake surface area and watershed area were determined on 1:50 000 topographic maps with a compensating polar planimeter.

2.3.2. Sample Collection:

Sediment cores were collected from the 34 study lakes designated for surface sediment sampling from August 14-20, 1984. All lakes were assessed by Bell Jet Ranger 206B helicopter and all coring operations were conducted from the floats of this aircraft. Coring sites were established over the point of apparent maximum depth (mid-lake) and

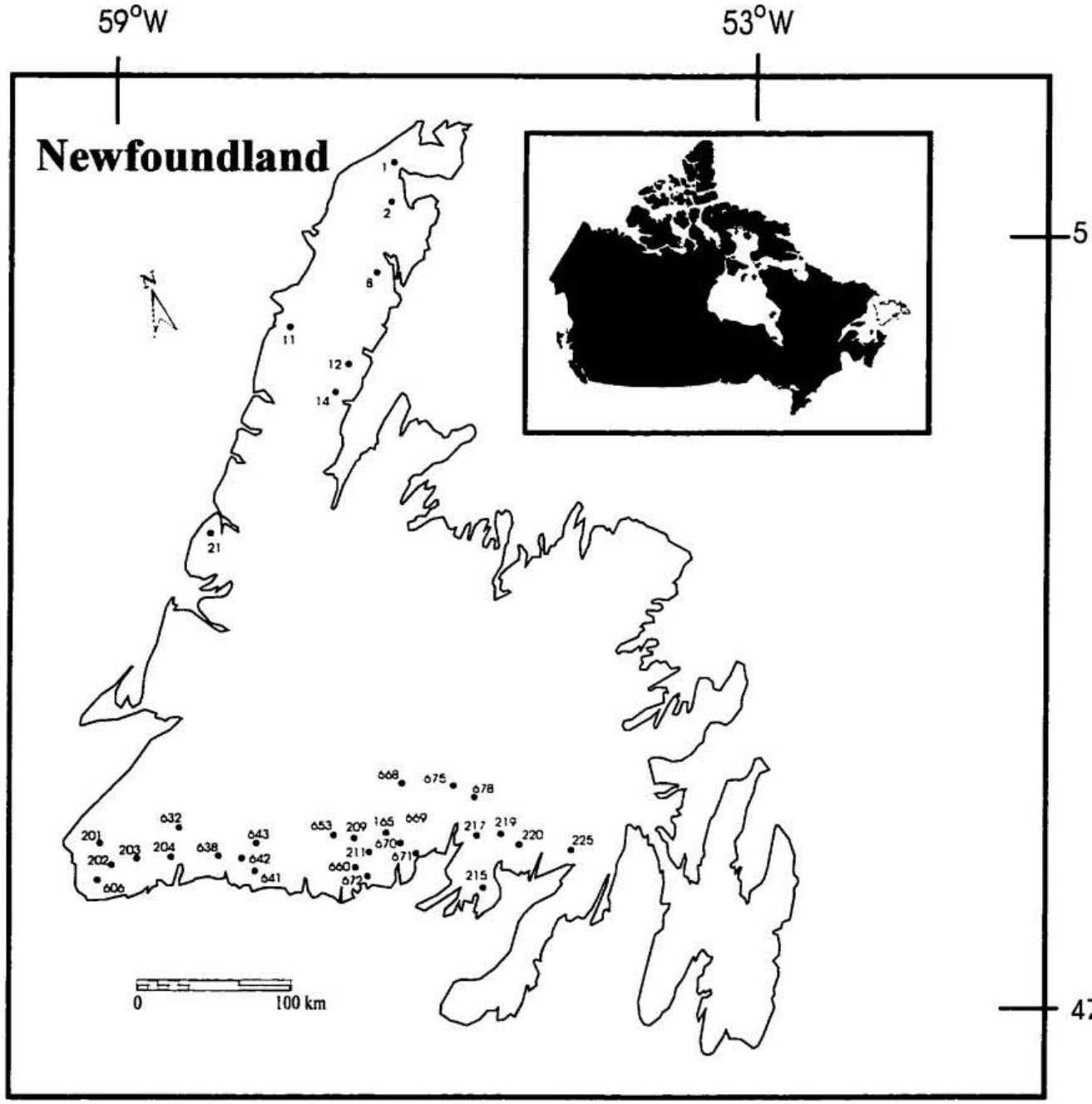

Figure 2.1.: Location of sampling sites.

the depth of the site was recorded. Cores were collected using a modified 10 cm diameter, light-weight Williams and Pashley (1979) corer, designed for use in unconsolidated deposits. One core per lake was obtained and all cores retained for analysis had an undisrupted sediment/water interface. There was no evidence of bioturbation in any of the cores collected. The top 1 cm horizon was removed from the core by spoon (if very watery sediment) or by spatula (in more consolidated sediment) and transferred to pre-labelled vinyl whirl-pak bags and frozen upon return to the field laboratory.

Water sample collection methods and variables analysed have been described by Scruton (1983). Initially, a water sampling station was established at or near the midpoint of each lake. Secchi depths were determined by lowering a 30.5 cm diameter Secchi disc. An Intersil AD 590 transducer permitted sounding of the sampling site. In shallow lakes (3 m or less), water was dipped 0.5 m below the surface. In all other lakes, a composite water sample was collected with a tube sampler in accordance with the Ontario Ministry of Natural Resources manual (1980). Samples for alkalinity and pH were stored in 500 ml linear polyethylene (LPE) Nalgene bottles. All samples were stored at 4^0C (degrees Celsius) until field analyses were completed or until samples were shipped to the analytical laboratory. Samples for Cl^{-1} (chloride) and SO_4^{-1} (sulfate) were collected in LPE scintillation vials (20 ml) and frozen upon arrival at the mobile field laboratory. Water samples were kept cool in insulated coolers and nutrient samples kept frozen during shipment during air shipment to the selected analytical laboratory.

2.3.3. Sediment Mercury and Water Analysis:

Sediment samples were analysed for mercury content by Atlantic Analytical Services Limited (P.O. Box 489, Springdale. NF Canada A0J 1TO). Homogenized sub-samples of the surface sediments were obtained and the wet weights recorded. Each subsample was then dried at 90^{0}C for at least 24 hours and the dried weight recorded. The dried sediment was then crushed and a subsample was ashed at 550^{0}C for two to three hours. The ashed sediment was then put into solution by digestion with HF-Aqua Regia acid solution. A Fisher model 1C (Industrial Laboratories) 951 Atomic Absorption Spectrophotometer was used to analyse the sediment solution for mercury using the cold vapour method (Environment Canada 1979), with a detection limit of 0.01 ppm. Internal standards and blanks (every 10th analysis minimum) were run as internal laboratory checks on quality assurance.

Lake water samples were analysed for pH and alkalinity at a mobile field laboratory within 24 hours of collection. A Fisher Accumet 119 portable digital pH meter accurate to 0.01 pH units was used for all field measurements of pH. The pH meter was calibrated daily prior to the start of field and laboratory routines using standard reference buffer solutions (pH 4.0, 7.0). Samples for alkalinity determination were stored, cooled, and then warmed to room temperature for analysis. Alkalinity was determined by Gran titration as follows: a 100 ml aliquot of sample was drawn off and transferred to a 250 ml beaker. After recording the initial pH, small amounts of $H_2SO_{4\ (aq)}$ (0.01 N) were delivered using a Canlab repipet (with an accuracy and reproducibility of ± 1%) and the resulting change in pH recorded. A minimum of 30 readings in the pH range of 5.5 to 3.5 were

obtained and alkalinity was calculated from the Gran titration according to a modified computer routine (Ontario Ministry of Natural Resources, 1980).

All methods used to determine the water sample parameters followed those outlined in Environment Canada (1979) and the American Public Health Association *et al.* (1975) and were carried out at the selected analytical laboratory. Conductivity was measured by a Radiometer Conductivity Bridge (CDM 2e) in micro siemens per centimetre ($\mu S\ cm^{-1}$). Sodium, potassium, calcium, and magnesium concentrations were determined by direct aspiration using atomic absorption spectrophotometry. Colorimetric determinations using a Technicon Auto Analyser were used for sulfate and chloride. Aluminum was determined by the Atomic Absorption - Heated Graphite Atomizer (HGA) method. Colour was determined by visual comparison with platinum colour plasma in Total Colorimetric Units (TCU). An EPA standard reference sample and three blind batches of lake water samples were also analysed by participating labs to permit an interlab comparison of data.

2.3.4. Data Analysis:

Chemical and physical data were run as Pearson correlates in combination with the sediment mercury levels determined at each lake. Any significant correlates ($\propto = 0.05$) were then included in linear regression analysis to estimate the functional relationship. Significance tests were not reported from regression analysis since non-significant correlates were removed prior to analysis. Regression p-values calculated on the preselected set would not be accurate estimates of Type I error. Statistical analysis was

performed with Sigma Stat Statistical Software Version 2 (Jandel Scientific, 1994) and Minitab Software Release 9.1 for VAX/VMS (1992).

2.4. Results:

The lakes selected were small in size, ranging from 6 to 1117 ha with relatively high order drainage systems (70% first order and 15% second order lakes). The watershed areas ranged from 6 to 6478 ha. The deepest lake was 23 m while the shallowest lake was only 1 m deep. Secchi transparency, collected for only 19 of the 34 lakes, ranged from 1 to 9.5 m. In three of the lakes, the Secchi Depth was equal to the maximum depth (Table 2.1). Lakes were generally low in elevation with a maximum value of 480 m (Table 2.1).

Lake pH ranged from 4.86 to 7.72 with 85% of the lakes having a pH of <7.0 (Table 2.2). Alkalinity values were generally low, ranging from -5 to 1715 μeq l^{-1} with a mean of 192.4 μeq l^{-1} (standard deviation of 435.3μe l^{-1}), indicating low buffering capacity. According to lake sensitivity criteria (Ontario Ministry of the Environment, 1981) 74% of the lakes were extremely acid sensitive (0-40 μeq l^{-1}), and 9% were moderately sensitive (41-200 μeq l^{-1}). Conductivity values were also generally low with a mean of 30.9 μS cm^{-1} and 71% of the lakes sampled produced values $\leq$ 20.0 μS cm^{-1} indicating the soft and dilute nature of much of Newfoundland's freshwaters. Scruton *et al.* (1991) distinguished insular NF lakes $\leq$15 TCU as clearwater lakes, lakes 15-50 TCU as coloured, and lakes >50 TCU as highly coloured lakes. Using this criterion, lakes sampled were generally coloured (62%) with a mean of 30.9 TCU (Table 2.2). Six (18%) of the lakes sampled were highly coloured. This is generally reflective of the heavily

Table 2.1.: Selected morphometric and physical characteristics of the 34 study lakes.

Lake No.	Surface Area (ha)	Watershed Area (ha)	Lake Order	WA:LA[1]	Apparent Maximum Depth (m)	Secchi Transparency (m)	Elevation (m
1	147	706	1	4.8	2.5	2.5	30.5
2	23	205	1	8.9	4.7	4.2	38.1
8	104	617	1	5.9	4.0	3.0	297.2
11	232	660	1	2.8	20.0	5.0	57.9
12	25	167	1	6.7	12.0	1.0	403.9
14	50	643	1	12.9	13.0	1.5	393.2
21	33	404	1	12.2	3.3	1.7	114.3
201	98	700	1	7.1	15.7	3.3	297.2
202	34	197	1	5.8	21.0	5.5	464.8
203	24	127	1	5.3	5.5	3.5	419.1
204	23	257	1	11.2	20.0	2.0	312.4
209	88	591	1	6.7	2.2	2.2	256.0
211	95	1209	1	12.7	3.3	2.5	280.4
215	87	508	1	5.8	23.0	3.8	131.1
217	244	1010	1	4.1	10.1	4.2	236.2
219	1117	6478	1	5.8	3.2	2.5	167.6
220	385	2180	2	5.7	5.2	4.7	175.3
225	116	577	1	3.5	9.5	9.5	205.7
253	37	231	1	6.2	20.0	3.5	283.5
606	20	146	1	7.3	2.0	n\t[2]	304.8
632	190	630	1	3.3	4.0	n\t[2]	480.1
638	60	190	1	3.2	15.0	n\t[2]	434.3
641	100	240	1	2.4	3.0	n\t[2]	358.1
642	110	406	1	3.7	12.0	n\t[2]	449.6
653	280	1202	5	4.3	2.5	n\t[2]	281.9
660	164	752	2	4.6	8.0	n\t[2]	266.7
665	26	308	2	11.8	1.0	n\t[2]	205.7
668	104	604	1	5.8	5.0	n\t[2]	251.5
669	238	554	1	2.3	1.0	n\t[2]	175.3
670	16	144	1	9.0	1.0	n\t[2]	236.2
671	6	15	2	2.6	1.0	n\t[2]	175.3

672	10	110	2	11.0	9.0	n\r²	312.4
675	88	376	3	4.3	1.5	n\r²	251.5
678	26	108	1	4.2	2.5	n\r²	190.5
Mean	129.4	683.9	1.3	6.3	7.8	3.5	262.9
Range	1111.0	6463.0	4	10.6	22.0	8.5	449.6
Std. Dev.[3]	196.4	1107.9	0.8	3.2	6.9	1.9	118.6

[1]watershed area to lake area ratio.
[2]not taken.
[3]standard deviation.

Table 2.2.: Chemical characteristics of the 34 study lakes from samples collected August 14 to 20, 1984.

Lake No.	Hg (ppm)	pH	Alkal[1] (μeq L^{-1})	Conduct[2] (μS cm^{-1})	Colour (TCU)	COOH^{-} (μeq L^{-1})	Ca^{+2} (μeq L^{-1})	Mg^{+2} (μeq L^{-1})	Na^{+} (μeq L^{-1})	K^{+} (μeq L^{-1})	Cl^{-} (μeq L^{-1})	SO_4^{-2} (μeq L^{-1})	A (
1	0.044	7.72	1715	198.0	10.0	41.7	1473	446	174	5	189	56	b
2	0.042	7.42	1326	149.0	10.0	61.3	1080	276	126	7	127	40	3
8	0.007	5.34	14	13.6	10.0	29.9	20	19	61	4	59	23	1
11	0.038	7.26	915	130.0	10.0	39.4	753	345	174	8	198	75	1
12	0.022	5.13	5	13.6	50.0	47.5	19	18	48	4	48	19	1
14	0.044	5.23	18	14.4	60.0	56.8	22	21	52	4	48	19	1
21	0.061	7.10	674	94.0	60.0	76.5	498	293	170	9	158	56	9
201	0.055	6.04	34	31.2	50.0	62.7	77	50	135	5	141	42	1
202	0.075	6.42	45	26.2	15.0	30.4	104	29	91	1	96	33	5
203	0.052	5.22	6	15.6	15.0	25.9	24	16	65	1	54	25	9
204	0.061	5.13	9	15.5	50.0	46.0	32	19	65	4	54	31	1
209	0.063	6.19	68	19.5	10.0	44.9	97	22	61	2	56	17	2
211	0.058	5.65	27	13.0	20.0	41.2	32	17	61	3	48	17	6
215	0.022	5.51	10	22.8	20.0	34.3	36	30	117	3	118	33	1
217	0.033	5.45	9	16.1	17.5	32.2	30	21	70	2	73	25	5
219	0.042	5.62	11	19.2	17.5	41.9	39	26	87	6	85	27	8
220	0.019	5.68	52	16.8	17.5	33.5	31	20	87	3	79	29	7
225	0.003	5.85	40	17.7	10.0	14.4	34	19	79	3	79	33	6
253	0.036	7.45	1222	131.0	15.0	49.4	833	420	87	5	87	60	2
606	0.005	5.37	32	23.7	87.5	107.7	75	39	70	5	70	54	2
632	0.022	5.46	5	13.5	15.0	24.6	24	17	56	3	56	27	4
638	0.030	5.30	32	12.5	17.5	23.9	22	15	48	2	48	27	8
641	0.028	4.94	25	14.6	30.0	39.2	17	14	45	1	45	38	2
642	0.013	5.31	13	11.3	15.0	20.6	18	13	45	2	45	19	4
653	0.047	5.81	23	13.0	17.5	32.3	41	13	42	2	42	31	6
660	0.042	5.46	11	13.6	15.0	30.6	26	16	56	3	56	19	6
665	0.156	5.18	-5	14.4	50.0	48.9	22	20	51	3	51	17	1
668	0.044	6.43	88	20.3	25.0	51.4	91	62	34	3	34	13	3
669	0.028	5.67	31	20.0	70.0	91.5	70	35	62	3	62	19	1
670	0.030	4.87	26	16.6	75.0	57.0	19	17	54	1	54	19	1
671	0.019	4.86	7	18.2	75.0	65.7	25	20	56	3	56	19	2
672	0.027	5.19	29	16.1	50.0	46.6	28	17	56	3	56	27	1

675	0.023	5.61	22	13.2	25.0	47.0	27	31	39	3	39	17	8
678	0.023	5.15	2	12.8	15.0	25.5	17	16	48	1	48	13	2
Mean	0.039	5.77	192.4	35.1	30.9	44.5	169.3	72.1	79.5	3.4	75.3	30.0	1
Range	0.153	2.86	1720	186.7	77.5	93.3	1456	433.0	135.0	8.0	164	62.0	2
Std. Dev.[4]	0.027	0.79	435.3	46.52	23.2	20.02	345.2	122.9	37.4	1.9	42.0	15.0	6

[1]alkalinity
[2]conductivity
[3]below determination
[4]standard deviation.

stained bog water in many NF headwater lakes.

Calcium (Ca^{2+}) levels were generally low with some exceptions (Lakes 1,2, 21, and 253; Table 2.2). Magnesium (Mg^{2+}) and sodium (Na^{+}) cation levels were variable with respective means (and ranges) of 72.1 (13 to 446 μeq l^{-1}) and 79.5 μeq l^{-1} (13 to 174 μeq l^{-1}) (Table 2.2). Potassium (K^{+}) levels in all lakes were low with a mean of 3.4 μeq l^{-1}. Chloride (Cl^{-}), sulfate (SO_4^{2-}), and aluminum (Al^{3+}) ion levels were variable (Table 2.2). Organic acid anion ($COOH^{-}$) levels ranged from 14.4 to 107.7 μeq l^{-1} with a mean of 44.5 μeq l^{-1}.

Mercury values observed in sediments had a mean of 0.039 ppm and a range of 0.003 to 0.156 ppm. Mercury levels above 0.75 ppm, the 'safe' level set by the Ocean Dumping Control Act (Wilson and Travers, 1976) were observed in only Lake #665 (Table 2.2, Figure 2.2).

Pearson correlation analysis indicated that mercury sediment levels were not correlated with acidity. Mercury in sediments were only significantly related to WA:LA (Table 2.3.; Figure 2.3). The $\log_{10}$ transformed sediment mercury values and $\log_{10}$ transformed WA:LA were also significantly correlated ($r=0.374$; $p= 0.0293$). As well, WA:LA was correlated with colour ($r= 0.366$, $p=0.0333$; Figure 2.4.) and negatively correlated with Secchi depth ($r=-0.5731$, $p= 0.0103$; Figure 2.4). Secchi depth and colour were negatively correlated ($r= -0.5378$, $p= 0.0175$). None of the other chemical and physical parameters investigated were significantly correlated with sediment mercury levels (Table 2.3.) including watershed area and lake area.

Table 2.3.: Pearson correlation coefficients for sediment Hg and other physical and chemical characteristics of the study lakes.

Variable	Pearson Correlation Coefficient	p-value	n
Lake Surface Area	-0.106	0.5770	34
Watershed Area	-0.0210	0.9124	34
WA:LA	0.5176	0.0034	34
Lake Order	0.0551	0.7725	34
Maximum Depth	-0.0601	0.7523	34
Secchi Depth	-0.0784	0.7649	19
Elevation	-0.0586	0.7584	34
pH	0.120	0.5290	34
Alkalinity	0.148	0.4430	34
Conductivity	0.0331	0.8622	34
Color	0.0850	0.6551	34
Sulphate	0.00681	0.9715	34
$COOH^-$	0.117	0.5360	34
Ca^+	0.0322	0.8657	34
Mg^{+2}	0.0315	0.8687	34
Na^+	0.0815	0.6685	34
K^+	0.0898	0.6368	34
Cl^-	0.0659	0.7295	34
Al^{+3}	0.0100	0.9588	33

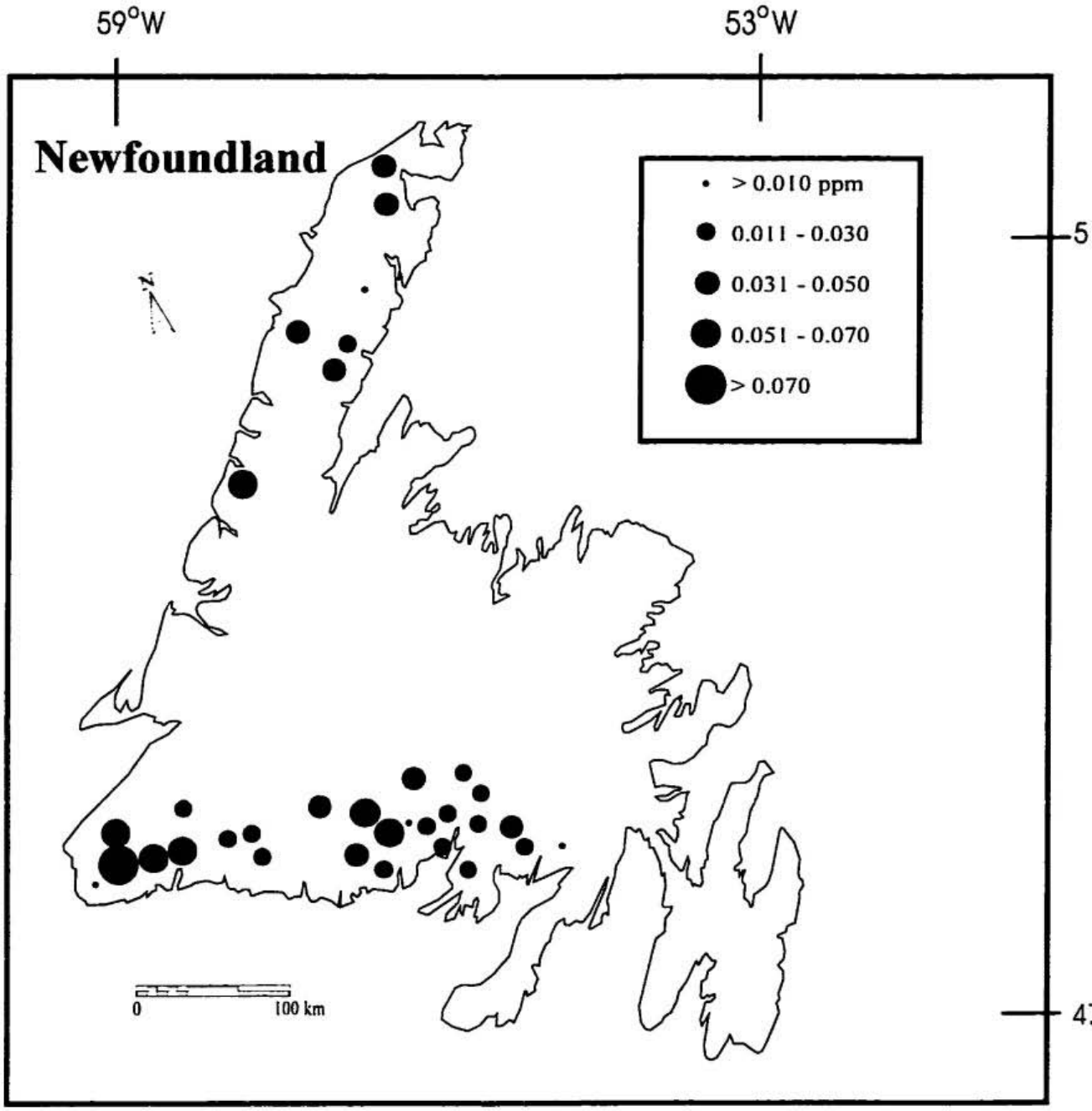

Figure 2.2.: Sediment mercury concentrations (ppm) in sampling sites.

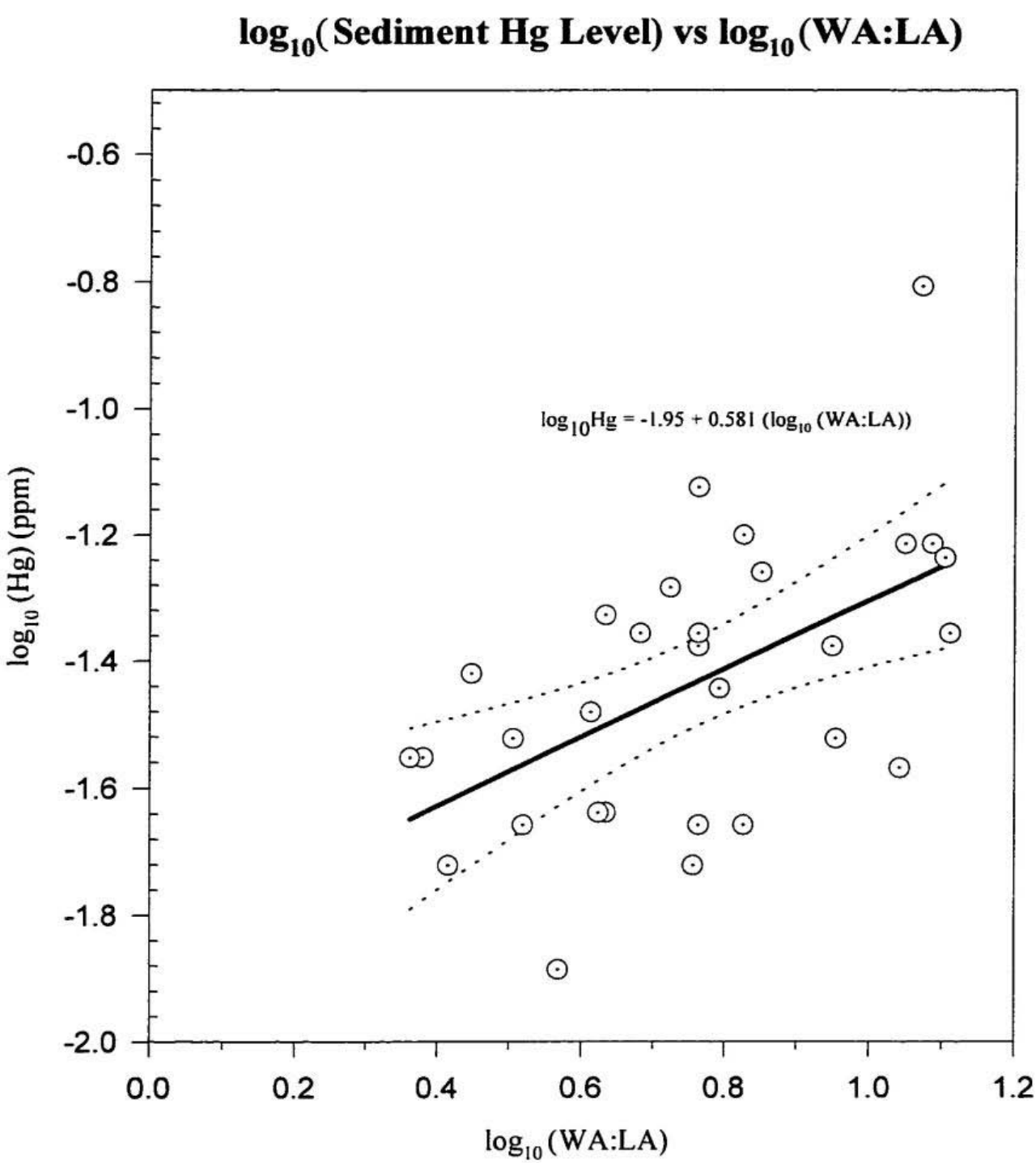

Figure 2.3.: Plot of log (sediment mercury levels (ppm)) vs log (Watershed Area: Lake Area) for the 34 study lakes with regression line. Dotted lines represent the 95% CI for the regression.

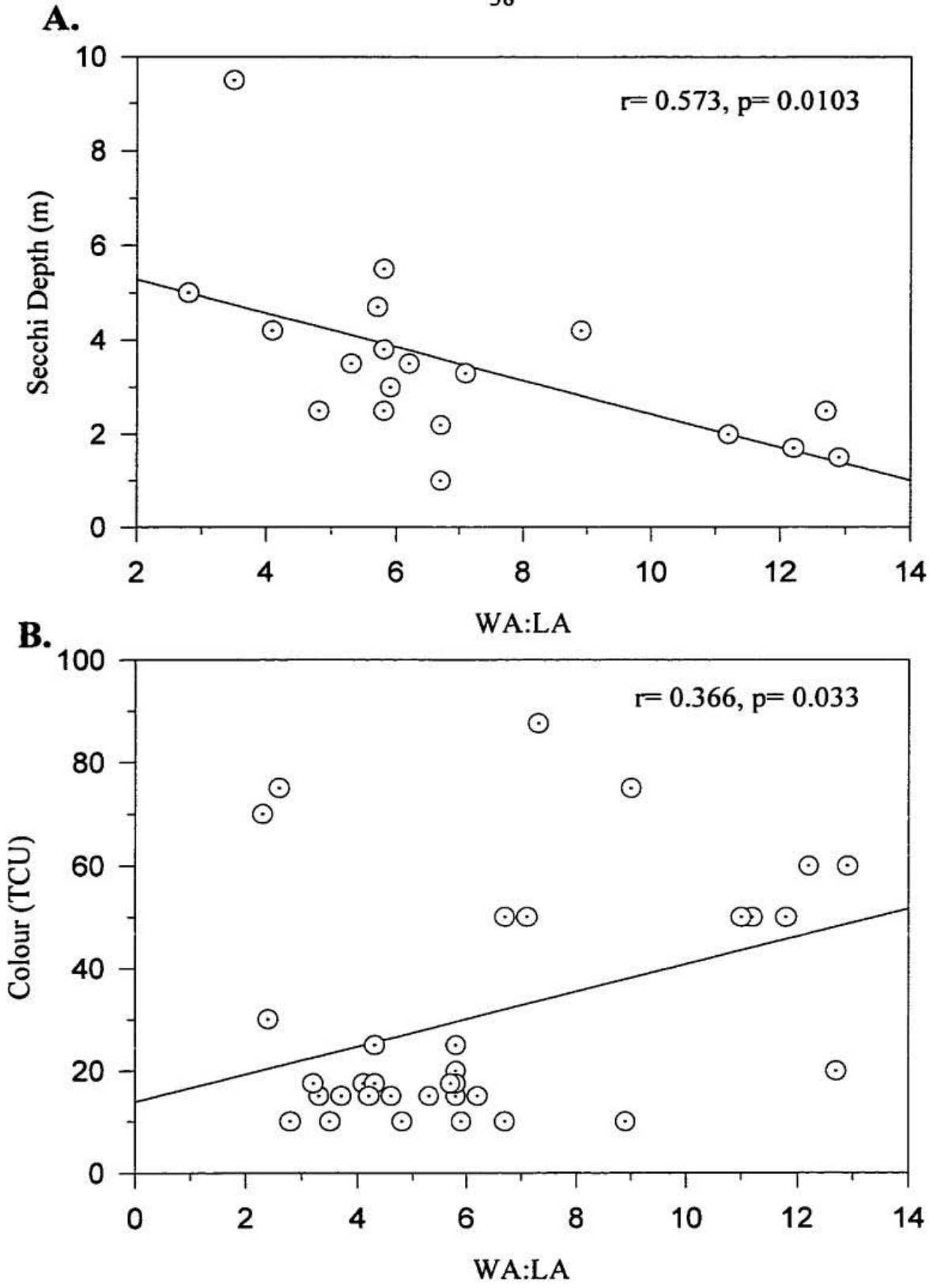

Figure 2.4.: Plots of (A) Secchi depth (m) versus WA:LA and (B) Colour (TCU) versus WA:LA. Solid lines represent the regression.

Linear regression of $\log_{10}$ (Hg) vs $\log_{10}$ (WA:LA) was used to estimate the relationship of sediment Hg to WA:LA (Figure 2.3). The log transformation was used to permit a better biological and physical interpretation of the model. The antilog transformation of this model relates sediment Hg to WA:LA as follows:

$$\text{Sediment Hg} = 0.01122\ (\text{WA:LA})^{0.581} \qquad \text{Equation 1.}$$

This equation suggests that sediment Hg is proportional to both LA and WA. However, as indicated earlier, sediment mercury was not significantly correlated with either lake area or watershed area alone.

2.5. Discussion:

In this among-lake comparison, acidity was not significantly related to sediment mercury levels. The lakes investigated ranged in pH from 4.86 to 7.72 and alkalinity values were insufficient to buffer the effects of acidification (Table 2.2). The effects of acidity on methylation in sediments has been extensively studied in aquatic systems (DeSimone *et al.*,1973; Miller and Akagi, 1979; Ramlal *et al.*, 1985; Steffan *et al.*, 1988; Gilmour and Henry, 1991), and acidity has been identified as an important factor affecting mercury sediment levels either directly or indirectly by influencing the dissolution or methylation processes. Lake water acidification can increase mercury concentrations in water by decreasing gross sedimentation rates, by mobilizing mercury from sediments, or by affecting sediment-water interactions in lakes (Verta *et al.*, 1990). The lack of correlation between the lake sediment mercury values and acidity indicated that lake pH was not affecting mercury concentrations in the bottom sediments of the study lakes. Acidification of Ontario soft-water lakes did not result in appreciable displacement of divalent mercury from bottom sediments by the action of H^+ ions (Jackson *et al.*, 1980) suggesting perhaps a more complex relationship between sediment mercury and acidity among lakes than within a single sediment type.

Sediment mercury was expected to vary with acidity. Atmospheric deposition of mercury can be in combination with ions associated with acid deposition, such as SO_4^{-2} or Cl^- (Pleijel and Munthe, 1995), suggesting a relationship between increased mercury concentrations and acidity in aquatic systems. The lack of correlation between sediment Hg and Cl^- or SO_4^{-2} may be explained by the close proximity of the ocean to many of the

study lakes. Chloride and sulphate levels may be representative of inputs of marine aerosols rather than inputs from acid rain (Kerekes and Hartwell, 1980; Sullivan *et al.*, 1988). A survey of 109 headwater lakes in insular Newfoundland in 1981 found that the relative contributions of chloride and sulphate to lakes from marine aerosols were 100% and 26% respectively (Scruton, 1983). Furthermore, microbial reduction of ocean sulphate to sulphide can interfere with and totally inhibit mercury methylation by forming insoluble mercury sulphide (Compeau and Bartha, 1983). This conversion may also be confounding the relationship between sediment mercury and acidity.

Lake area and watershed area were not significantly related to sediment mercury levels in this study. In contrast, McMurty *et al.* (1989) found that mercury levels in lake trout (*Salvelinus namaycush*) were positively correlated with lake area indicating that lake size may influence the availability of mercury in an aquatic ecosystem. Only WA:LA was significant in explaining the variation observed in surface sediment mercury levels. Equation 1 suggested that sediment mercury increased as watershed area increased or as lake area decreased. However, this was not confirmed by the analysis indicating that sediment mercury depends on the ratio between watershed area and lake area. This indicated that a large watershed may be depositing significant amounts of Hg sorbed to organic material to small lakes. These small lakes may then act as sinks, accumulating mercury in bottom sediments.

The province of Newfoundland is dominated by extensive areas of organic deposits, having in excess of 2 million ha of peatlands; surface waters tend to be highly colored, reflecting the occurrence of organic deposits (Rybak *et al.*, 1989). Large

watersheds characteristically high in organic matter, either in the form of peat or humus, may be depositing larger amounts of Hg coupled to organic matter to the lake bottom. Mierle and Ingram (1991) found that the close correlation of Hg with color suggested that humic material mobilized Hg and that the role of the watershed in controlling the loading of Hg to lakes should be explainable by the export of humic matter.

WA:LA was correlated with color and Secchi Depth. Color reflects the amount of organic acids dissolved in the water column (Scruton, 1983; Wetzel, 1983), and secchi depth, though linked to productivity, is probably more a function of color due to the oligotrophic and dystrophic nature of many of Newfoundland's freshwater lakes (Earle *et al.*, 1987). Mercury tightly couples to biogenic matter in all compartments of the biogeochemical cycle (Meili, 1991). Variation in the amount of Hg loading to lake sediments may arise from differences in the size of the watershed relative to the lake and differences in water color. Since NF watersheds are typically high in organic material, large watersheds can effectively color the lake system, especially where the lake is small. Increased color reflects the potential for significant input of mercury coupled to organic matter to a lake system from the watershed.

2.6. Conclusions:

Acidity did not affect mercury accumulation in lake sediments. Sediment mercury levels were found to be related to WA:LA rather than acidity. This suggested that large watersheds can deposit significant amounts of Hg sorbed to organic material to small lakes that then act as sinks for this metal.

2.7. Acknowledgements:

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Chapter 3

Temporal Variation of Mercury in Recreational Fish from Freshwater Reservoirs in NF, Canada.

3.1. Abstract: Mercury levels in fish generally increase after impoundment. This represents a potentially serious problem because of its bioaccumulation and its toxicity. Return time is hypothesized to be dependent on several factors, including the trophic status of fish and certain physical and chemical characteristics of the reservoir. A study in Newfoundland has predicted return times on the order of 10-12 years for landlocked Atlantic salmon (*Salmo salar*) and 7 years for brook trout (*Salvelinus fontinalis*).

Mercury in fish was measured in a number of Newfoundland hydroelectric reservoirs of various age (9-95 years) and size. The slope of the relationship between mercury concentration and fork length for salmonids was found to change significantly over time and was used to evaluate the temporal variation of mercury in reservoir fish. Reservoir age and conductivity were found to be significant in predicting the slope of the relationship between fish mercury levels and fork length for both ouananiche and brook trout. Slopes for arctic charr (*Salvelinus alpinus*) were a function of the per cent area flooded (Flooded Area / Reservoir Area). Slope variability between impounded and non-impounded mercury data precluded determination of return times for either species because of difficulty determining what represented a non-impounded slope.

An investigation of mercury time series data suggested that following impoundment, smaller fish had relatively high mercury concentrations. This lowered the slope during the first 3 to 4 years following inundation. Because fish from a specific younger age class (ie. 1+ years) accumulate mercury more rapidly than larger fish it is more efficient to monitor these younger fish to establish the impact of inundation on mercury accumulation.

Weighted regression indicated that the slope coefficient for the decline in mercury was 8 to 73 times lower than that for the early increase in post-impoundment mercury. Determination of what comprises a pre-impoundment level for reservoirs for which no pre-impoundment data exists is problematic, and the available data indicated that each site may have its own pre-impoundment level. Return times may be unique to each reservoir requiring that pre-impoundment mercury levels be measured in order to predict return times.

3.2. Introduction:

During the last 20 years, elevated mercury levels have been reported in fish from hydroelectric reservoirs while fish in unimpounded adjacent lakes have not shown such signs of increased mercury burden. This mercury is thought to have been released from the soil by inundation (Bodaly *et al.*, 1984; Hecky *et al.*, 1987; Morrison and Thérien, 1991; Montgomery *et al.*, 1995). Mercury concentrations in reservoir fish increase by factors of two to nine times over pre-impoundment concentrations or background concentrations in fish from unimpounded lakes in the same region (Bodaly *et al.*, 1997). After inundation, mercury levels in the fish rise and then decline toward pre-impoundment levels as the reservoir ages (Johnston *et al.*, 1991).

Due to the toxicity of mercury, it has become a goal to predict the time required for mercury in reservoir fish to return to pre-impoundment levels (Brouard *et al.*, 1991; Verta *et al.*, 1986; Jackson *et al.*, 1988; Verdon *et al.*, 1991; Scruton *et al.*, 1994). Estimates of the return time are quite variable, ranging from 3-5 years in Northern Manitoba reservoirs (Jackson, 1988) to 20-30 years in Quebec (Verdon *et al.*, 1991) and Finnish Reservoirs (Verta *et al.*, 1986). Observed return times for Northern pike (*Esox lucius*), were 11, 15, 20, and 22 years while seven other reports for this species were for greater than 15-22 years (Bodaly *et al.*, 1997). In Newfoundland, Canada, return periods of 7 to 12 years have been predicted for salmonids in recently flooded hydroelectric reservoirs (Scruton *et al.*, 1994).

Models predicting fish mercury levels are usually based on factors such as area flooded and reservoir age. Johnston *et al.* (1991) used an exploratory analysis

incorporating several physical characteristics of reservoirs to identify predictors of fish mercury levels. They found a strong relationship between the per cent flooded, the rise in surface level, and the mercury content of fish (Johnston *et al.*, 1991). Many other factors have been postulated to affect return times, including productivity, pH, alkalinity, water color, and DOC (Table 3.1.) which either stimulate or inhibit methylation (Wren and MacCrimmon 1983; Verta *et al.* 1986; McMurty *et al.* 1989; Wiener *et al.* 1990)

Methyl mercury is the form most readily accumulated by and found in biota, specifically fish. Mercury methylation has been considered primarily a biological process (D'Itri *et al.*, 1971; Bodaly and Hecky, 1979; Compeau and Bartha, 1985; Ramlal *et al.*, 1985) occurring within aquatic systems via bacterial methylation of inorganic mercury. Processes affecting mercury methylation in newly impounded reservoirs may influence mercury concentrations in fish and subsequently the return time for a reservoir. Stimulation of mercury methylation associated with increased productivity from the addition of organic compounds (Akagi *et al.*, 1979) in the flooded area may increase the availability of mercury to fish. Because methylation has been linked to lake productivity (Fagerström and Jernelöv, 1972; Gilmour, 1992), productivity measures may explain some of the variation seen in mercury return times from reservoir fish populations.

Elevated methyl mercury levels in fish from acidic lakes suggest a link between the process of mercury methylation, acid deposition (Gilmour and Henry, 1991), and return times. Survey data indicated that Hg concentrations in fish of a given species and age are often inversely correlated with lake pH or alkalinity (Wiener *et al.*, 1990).

Watras *et al.* (1995) found that high DOC favoured mercury methylation in a

Table 3.1.: List of abbreviations with name and symbol (units).

Name	Symbol (units)
Acidity	pH
Age Reservoir	AR (years)
Analysis of Variance	ANOVA
Area Flooded	AF (ha)
centimetres	cm
Chlorophyll a	Chl ($\mu g\ l^{-1}$)
Conductivity	Cond ($\mu S\ cm^{-1}$)
degrees Celsius	^{o}C
Department of Fisheries and Oceans	DFO
Dissolved oxygen	DO ($mg\ l^{-1}$)
Dissolved nitrogen	Nitrates ($\mu g\ l^{-1}$)
Fork length	FL (cm)
Grams	g
Hectares	ha
Hydrochloric acid	HCl
Litres	l
Mercury	Hg
meters	m
Newfoundland and Labrador Hydro	NFLH
N	normal
N-1- naphthyethylenediamine dihydrochloride	NNED
Particulate organic nitrogen	PON (μg)
Particulate organic carbon	POC (μg)
Per Cent Area Flooded	% Area Flooded
Phenyl Arsine Oxide	PAO
Secchi depth	Secchi depth (m)
Total phosphorous	TP ($\mu g\ l^{-1}$)
Revolutions per minute	rpm
Versus	vs

clear-water seepage lake. Driscoll *et al.* (1995) found that total mercury increased with increasing concentrations of DOC, while others found that decreased DOC increased net methyl mercury production in the water column (Winfrey and Rudd, 1990; Miskimmin *et al.*, 1992). These results appear somewhat contradictory, but it may be that physical and chemical characteristics of reservoirs that affect methylation will in turn, affect mercury levels in reservoir fish and return times.

Models predicting mercury return times have been based on physical characteristics of reservoirs such as the age (Abernathy and Cumbie, 1977; Verdon *et al.*, 1991; Messier *et al.*, 1995) and the degree of inundation (Johnston *et al.*1991). However, other factors associated with increased levels of mercury methylation and increased mercury burdens in fish have rarely been considered. Mercury concentrations in lake trout *(Salvelinus namaycush)*, smallmouth bass *(Micopterus dolomieui)* (McMurty *et al.*, 1989), sunfish *(Lepomis gibbosus)* (Wren and MacCrimmon, 1983), and walleye *(Stizostedion vitreum)* (Wiener *et al.*, 1990) have been linked to physical and chemical characteristics of lakes.

The goal of this study was to develop predictions of mercury return times in fish from hydroelectric reservoirs on the Island of Newfoundland. Existing predictions incorporated only the age of the reservoir (Scruton *et al.*, 1994). Therefore, models that incorporated physical, chemical, and biological characteristics of the reservoirs were tested in an attempt to account for more of the variation observed in fish mercury levels and to improve predictions.

3.3. Material and Methods:

3.3.1. Sampling Locations:

Reservoirs (Figure 3.1) were chosen based on age and size criteria (Table 3.2). Control ponds had no history of impoundment. Six of the reservoirs (Great Burnt Lake, Cold Spring Pond, Long Pond, Cat Arm, Granite Lake, Meelpaeg Lake) and two of the control ponds (Eclipse Pond, Rocky Pond) included in this study are part of a continuous monitoring program incorporated by DFO and NFLH since 1982 (Table 3.2). The remaining 9 reservoirs plus one control pond have not been monitored.

3.3.2. Water Sampling:

Water samples were taken during the summer of 1995 at the centre of each water body utilizing a Nisken bottle, then placed in an acid-washed collection vessel. All samples taken were analysed for DO, POC, PON, nitrates, TP, and Chl (Table 3.1).

DO was determined by the Winkler titration (Hach Water Handbook, 1973). Water was taken 1 m below the surface at each site and control pond, then held in clean 300 ml glass stoppered BOD bottles. Bottles were allowed to overflow to ensure no trapped air bubbles. One manganese powder pillow and one alkaline iodide-azide powder pillow were added to the sample, which caused a heavy yellow precipitate to form (an indicator of oxygen presence). The stopper was then inserted, and the bottle was inverted several times and placed in the dark. No more than 24 hours later, the contents of one sulfamic acid powder pillow was added to the sample. The stopper was replaced and the bottle shaken until all of the precipitate had dissolved. A 200 ml sample was then

Table 3.2.: Age and other characteristics of sampling sites; BT= brook trout, OU= ouananiche, AC= arctic charr.

Site	Position	Site #	Data Used (yr)	Age (yrs)	% Area Flooded	Area Flooded (ha)	Mean Depth (m)	No. of each Species Collected		
								BT	OU	AC
Middle Gull Pond (control)	47° 21.3' N 53° 18.3' W	1	1995	—	0	—	Unknown	26	26	0
Eclipse Pond[1] (control	48° 60.0' N 56° 46.8' W	2	1995	—	0	—	Unknown	20	26	0
Rocky Pond[1] (control)	48° 20.0' N 56° 00.0' W	3	1987	—	0	—	Unknown	40	40	0
Cochrane Pond	47° 27.9' N 52° 51.5' W	4	1995	85	60	30	2.4	21	21	0
Bay Bull's Big Pond	47° 24.7' N 52° 47.3' W	5	1995	95	54	175	7	20	0	0
Mobile First Pond	47° 15.3' N 52° 54.5' W	6	1995	44	23	40	Unknown	20	21	0
Mobile Big Pond	47° 15.9' N 52° 59.9' W	7	1995	55	69	378	8	27	23	0
Mount Carmel Pond	47° 08.9' N 53° 04.7' W	8	1995	42	24	140	9.5	30	14	0
Trinity Pond	48° 24.6' N 53° 27.8' W	9	1995	40	16	95	4	0	17	30
Sandy Lake	49° 14.7' N 56° 59.6' W	10	1995	32	8	75	2	27	22	0
Hind's Lake[1]	48° 59.7' N 57° 03.1' W	11	1995	16	46	2141	Unknown	14	31	0
Red Indian Lake	48° 48.1' N 56° 34.2' W	12	1995	86	Unknown	Unknown	Unknown	17	18	5
Joe Dennis Pond	48° 23.0' N 58° 12.3' W	13	1995	50	7	21	4.8	26	0	0
Great Burnt Lake[1]	48° 19.6' N 56° 14.2' W	14	1990	8	45	2500	10	28	26	0
Cold Spring Pond[1]	48° 11.4' N 56° 17.4' W	15	1992	10	41	1000	8	48	32	10
Long Pond[1]	48° 05.5' N 55° 49.5' W	16	1992	26	62	13000	16	33	83	22
Cat Arm[1]	50° 04.0' N 56° 56.5' W	17	1993	9	82	4300	18	103	0	143
Cat Arm '82	50° 04.0' N 56° 56.5' W	0	1982	0	0	——	Unknown	29	0	27
Granite Lake	48° 10.5' N 56° 59.2' W	18	1994	24	Unknown	8000	Unknown	10	36	25
Meelpaeg Lake	48° 19.6' N 56° 14.2' W	19	1994	26	86	15900	Unknown	0	34	27

[1]Time series available.

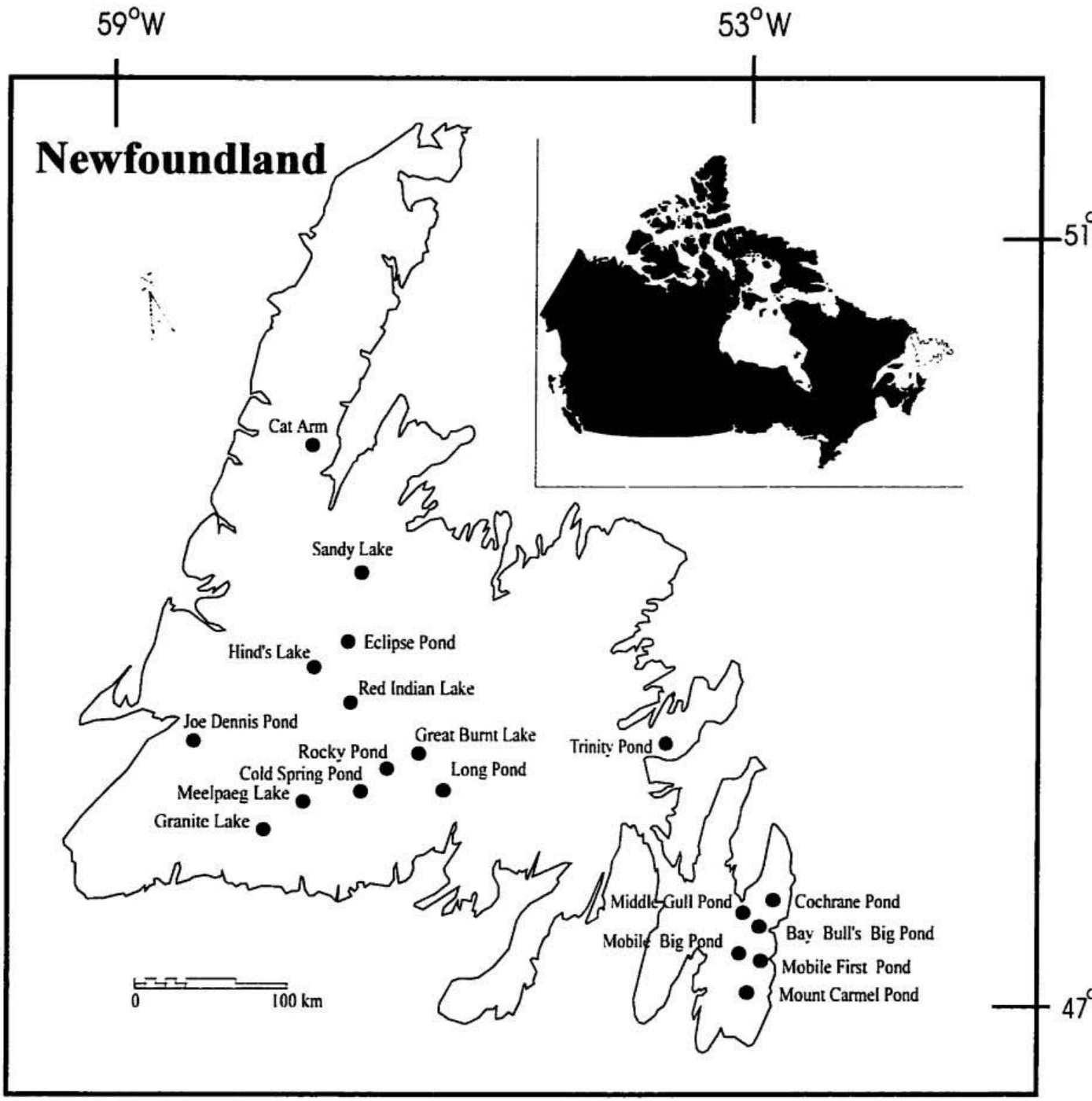

Figure 3.1.: Location of sampling sites on the Island of Newfoundland.

transferred to a 250 ml Erlenmeyer flask. This solution was titrated with standard PAO solution (0.025 N) to a pale yellow color. Two dropperfuls of starch indicator solution were then added turning the solution dark blue. The titration continued until the solution turned colorless. The total number of ml of PAO solution is equal to the number of mg l^{-1} of dissolved oxygen in the sample. Two BOD bottles were filled at each site and control pond. Titrations were run in triplicate and the average of the last two titrations were taken.

Total phosphorous was determined with a modification of Murphy and Riley (1962). Samples were taken at 1 m depth from each site and control pond, filtered through glass fibre precombusted filters, and collected in 125 ml acid washed sample bottles. Samples were frozen until analysis. Standards were prepared ranging from 10 to 180 μg ml^{-1} and were run several times prior to samples to ensure repeatability. Reagents were prepared according to Stainton *et al.* (1977). Potassium dihydrogen hydride phosphate was used to make phosphate standards. Twenty-five ml of each standard was placed in a glass 75 ml stoppered scintillation vial and 0.65 g of potassium persulfate was added. This was then autoclaved for 20 minutes. After autoclaving and the solutions had cooled, 5 ml of mixed molybdate reagent was added. Standards were then transferred to a 10 cm path cuvette and the absorbances read at 885 nm with a spectrophotometer. Absorbance was then plotted against standard concentration. Samples and standards were prepared in the same manner. Sample phosphorous concentrations were determined from a standard curve in which absorbance was plotted against standard concentration. Standards and blanks (glass distilled deionized water) were run at the beginning and end

of analysis. Samples from sites and control ponds were run in duplicate and the average absorbance was taken.

Nitrates were determined with cadmium reduction outlined in Stainton *et al.* (1977). Samples were taken at 1 m depth from each site and control pond, filtered through glass fibre precombusted filters, and collected in 125 ml acid washed sample bottles. Samples were frozen until analysis. Standards ranged from 0.01 μg ml^{-1} to 0.13 μg ml^{-1} and were prepared from a potassium nitrate stock solution. Nitrate standards were run in duplicate on each reducing column used. Fifty ml aliquots of each standard was added to each column and allowed to drip through at a rate of approximately 0.01 ml s^{-1}. The first 10 ml of effluent was discarded and the next 25 ml collected in a graduated cylinder. Five hundred μl of sulphanilamide solution was added to the effluent and mixed. After 5 minutes, 0.5 ml of NNED (Table 3.1.) was added to the effluent and mixed. Between 10 minutes and 2 hours later the absorbance was read at 543 nm. A 10 cm path cuvette was used to determine the absorbances of samples and standards. Samples were prepared in the same manner as standards. A distilled deionized water blank was carried through each column at the beginning and end of the procedure. As well, a standard was run following blanks. A unit extinction factor was determined for the 10 cm light path equal to the standard concentration divided by the absorbance of standards plus reagents. The concentration of nitrate in samples was calculated by multiplying the sample absorbance by the extinction factor. Samples from sites and control ponds were run in duplicate and the average absorbance taken.

POC and PON were both determined with a Perkin-Elmer 2400 CHN analyser.

Water samples (200 ml) were collected in 250 ml vinyl chloride bottles and kept in ice. Samples were filtered onto precombusted glass fibre filters within 12 hours of collection. Following filtration, filters were removed with forceps and placed on tinfoil squares. Filters and tinfoil squares were both dried in an oven at 55^{0}C for two days. Following drying, the filters were wrapped in the tinfoil squares and together they were compressed into pellets. Pellets were then placed in a covered dish with label. Blanks consisting of a tinfoil square and filter were run as every 10th sample and were placed in front of and behind standards. Standards (2 mg acetanilide + filter + tinfoil) were run as the second and second last sample in the collection. The CHN analyser reports the amount of POC and PON and together they were used to determine C:N (Table 3.1).

Chlorophyll was filtered onto glass fibre filters in the same manner and in the same amount as samples for CHN analysis. Filters were also frozen in scintillation vials following filtration and Chl was measured using a fluorometer in accordance with Strickland and Parsons (1972). Acetone (5 ml) was delivered to vials containing filters with a Canlab repipet then shaken. Vials were then placed in a freezer at -30^{0}C. After 24 hours vials were taken from the freezer and shaken. All fluid in the vial was transferred to a centrifuge tube. Vials were capped and rinsed twice with cold acetone, adding the fluid to the centrifuge tube each time. The volume in the centrifuge tube was then recorded as the volume extracted. The tubes were centrifuged at 3000 rpm for 5 minutes and the volume in each tube transferred to a cuvette. The fluorometer was zeroed with an acetone blank and the gain of the appropriate sample recorded as F_0. One to 2 drops of 5% HCl were added to the cuvette and the gain was recorded again (F_a). The fluorometer was

zeroed after every reading and the appropriate gain was used to get a reading of F_s above 25 and a reading of F_0 below 1200. The Chl concentration in the sample was then calculated as $\mu g\ l^{-1}$ using the calibration factor for the fluorometer, the volume filtered, and the ΔF (Strickland and Parsons, 1972).

All other variables, including pH, conductivity, and Secchi Depth were determined directly in the field. Acidity was determined with a Hanna Instruments Piccolo portable pH meter with automatic temperature compensation and precision to ± 0.01 pH units. Conductivity was determined with a Hanna Instruments HI 8733 portable conductivity meter with automatic temperature compensation and a resolution of 0.1 $\mu S\ cm^{-1}$. Meters were cleaned and calibrated prior to each field trip. Secchi depth was determined visually by the same person with a 29.7 cm diameter secchi disk. Water samples from Rocky Pond were not collected.

3.3.3. Fish Species:

There are three main species of recreational fish in Newfoundland. These are landlocked Atlantic salmon or ouananiche (*Salmo salar*), brook trout (*Salvelinus fontinalis*), and arctic charr (*Salvelinus alpinus*). Fish were collected from 9 previously unsampled reservoirs and 1 control pond during the fall of 1995. A minimum of thirty individuals of each species of recreational fish present at a sampling site were collected according to a length stratification employed in the ongoing NFLH/DFO monitoring program (Table 3.3). This length stratification was based upon an arbitrary division of the maximum fork length obtained for the particular species into 6 size classes. In cases

where all size classes could not be filled, class numbers were compensated with fish from adjacent classes that were within 5 cm of the desired size. All fish sampled were sized (fork length (cm)) and identified to species. A fillet was taken from the left dorsal region of each specimen and frozen for subsequent mercury analysis. In the case of individuals less than 10 cm, the entire fish was frozen. Analysis for mercury was carried out at the Department of Fisheries and Oceans Inspection Laboratory using cold vapor atomic absorption spectrophotometry with a detection limit of 0.01 ppm. This data, compiled with that collected by DFO and NFLH using similar methods, has formed a comprehensive database of 16 reservoirs and 3 control ponds.

Table 3.3.: Length stratification utilized for fish collection in the reservoirs and control sites included in this study.

Length Class	Number Sampled	Species		
		Ouananiche	Brook Trout	Arctic Charr
A	5	<100 mm	<100 mm	<120 mm
B	5	101-200	101-140	121-150
C	5	201-300	141-180	151-180
D	5	301-400	181-220	181-210
E	5	401-500	221-260	211-240
F	5	>501	>261	>241

3.3.4. Data Analysis:

Slopes were determined from the regression of log (Hg (ppm)) vs log (FL (cm)) for each species by sample site and control pond. Mercury and fork length data were logarithmically transformed (log_{10}) to meet the statistical requirements for parametric analysis of normal residuals with homogeneous variance. Slopes obtained for each species by sample site and control pond were used as Pearson correlates in combination with physical, biological, and chemical characteristics of each site and control pond. Any significant ($\propto = 0.05$) characteristics were incorporated in multiple regression analysis to develop a model predicting the observed slope from the regression of log_{10} (Hg) vs log_{10} (FL) for each species. Factor were retained in the regression model using a screening criterion of $p = 5\%$ or less.

3.4. Results:

3.4.1. Sampling Locations:

Reservoirs sampled ranged in size rom 21 to 15 900 ha and in age from 9 to 95 years. The % Area Flooded of the reservoirs ranged from 7% at Joe Dennis Pond to 86% at Meelpaeg Lake (Table 3.2). Three control ponds were initially selected, two of which were sampled in 1995 (Table 3.2).

3.4.2. Water Samples:

Light penetration measured as Secchi depth ranged from 10 m at Middle Gull Pond to 2.3 m at Sandy Lake (Table 3.4). The acidity of the sampling sites ranged from 5.52 to 7.46 and was fairly low with a mean pH of 6.52; conductivity ranged from 10.1 μS cm^{-1} at Eclipse Pond to 55.2 μS cm^{-1} at Cochrane Pond. Nitrate values ranged from 0.9 $\mu g\ l^{-1}$ to 30.5 $\mu g\ l^{-1}$. TP levels were near or below the detection limits of the method employed in this study. DO ranged from 8.5 to 10.03 mg l^{-1} with a mean value of 9.12 mg l^{-1}. Chlorophyll levels ranged from 0.0859 to 0.292 $\mu g\ l^{-1}$; POC ranged from 0.0683 to 0.2218 $\mu g\ ml^{-1}$. C:N ranged from 7.43 to 20.23.

Table 3.4.: Physical and chemical characteristics determined for each of the sampling sites with summary statistics.

Site[1]	Secchi Depth (m)	Depth at Station (m)	pH	Conduct ($\mu S\ cm^{-1}$)	Nitrates ($\mu g\ l^{-1}$)	TP ($\mu g\ l^{-1}$)	DO ($mg\ l^{-1}$)	Chl ($\mu g\ l^{-1}$)	POC ($\mu g\ l^{-1}$)	P (μ
Middle Gull Pond	10	10	7.46	26.8	2.2	<10	9.55	0.0876	221.8	2.
Eclipse Pond	3.75	4.9	5.83	10.1	1.2	<10	10.03	0.2417	506.3	4
Cochrane Pond	3.53	3.53	7.32	55.2	1.08	11	8.63	0.1919	608.3	6
Bay Bull's Big Pond	4.5	14.86	7.15	53	7.5	<10	9.48	0.2706	363.0	2
Mobile First Pond	5.83	5.83	6.58	25	12	<10	9.23	0.1247	299.0	2
Mobile Big Pond	4.5	10.51	6.83	24.4	29.9	<10	8.95	0.0951	334.5	4
Mount Carmel Pond	4.2	16.37	6.48	25.8	0.9	10	9.17	0.2919	474.5	4
Trinity Pond	3.35	6.65	6.37	37.5	1.16	<10	8.83	0.2425	455.5	4
Sandy Lake	2.3	12.7	6.39	22.1	30.1	<10	9.00	0.1561	561.5	2
Hind's Lake	3	10	6.16	14.1	29	<10	9.23	0.1979	340.0	2
Red Indian Lake	N/D[2]	N/D[2]	6.45	21.3	30.5	<10	9.45	0.1127	326.3	2
Joe Dennis Pond	3.8	6.12	6.5	23.2	27.8	<10	8.50	0.1719	403.8	2
Great Burnt Lake	3.8	15.75	6.61	13.6	27.7	22.7	9.15	0.2003	333.8	3
Cold Spring Pond	3.5	16.3	6.5	14.5	29.8	<10	8.80	0.1896	438.3	4
Long Pond	4.5	22.55	6.47	14.1	30.4	<10	9.20	0.0859	280.5	1
Cat Arm	2.5	70	5.52	15	21.4	17.5	8.65	0.1705	455.8	3
Granite Lake	3.0	14.85	6.2	13.6	26.1	<10	8.85	0.1061	315.8	2
Meelpaeg Lake	3.5	6.05	6.48	13.2	29.0	23	9.45	0.2096	410.8	3
Mean	4.09	--	6.52	23.47	18.76	16.84	9.12	0.175	396.0	3
Std. Dev.	1.74	--	0.47	13.08	12.78	6.20	0.39	0.0633	102.0	1
Range	7.70	--	1.94	45.1	29.6	13.0	1.53	0.206	387.0	4

[1]No data for Rocky Pond.
[2]Not determined.

3.4.3. Fish Collection:

Not all fish species were found in all of the water bodies in this study. Sixteen of the 19 sites in this study had populations of ouananiche, 17 had brook trout, and only 7 had arctic charr (Table 3.2). Red Indian Lake only produced 5 charr of a similar size and so was not included in the analyses of Hg vs fork length for this species. None of the three control ponds produced arctic charr so pre-impoundment data from Cat Arm 1982 was used. Pre-impoundment data for brook trout from Cat Arm '82 were also included in analysis. The modal fork length observed for ouananiche was 30 cm which was close to the mean of 26.6 cm; the modal lengths for brook trout and arctic charr were 20 and 25 cm respectively which again were close to the respective means of 18.5 and 19.0 cm.

3.4.4. Mercury in Fish:

Ouananiche with mercury body burden in excess of the Canadian Safety Limit of 0.5 ppm were collected in 15 of the 16 sites including the three control ponds; brook trout in 8 of 17 sites including 2 controls; arctic charr in 3 of 6 sites (Table 3.5). Cat Arm preimpoundment data indicated no charr or brook trout over the Canadian Safety Limit.

Table 3.5.: Summary statistics for mercury levels observed by species and by sampling site.

Reservoir	Site	Species	n	Mean Hg (ppm)	Min. Hg (ppm)	Max. Hg (ppm)	Standard Deviation	n>.0.5 ppm (%)
Middle Gull Pond	1	brook trout	26	0.1538	0.11	0.36	0.0663	0 (0)
		ouananiche	26	0.3750	0.10	0.74	0.1931	6 (23)
Eclipse Pond	2	brook trout	20	0.1425	0.05	0.63	0.1296	1 (5)
		ouananiche	26	0.2696	0.07	0.68	0.1679	4 (15)
Rocky Pond	3	brook trout	40	0.1990	0..09	0.98	0.1403	1 (3)
		ouananiche	40	0.3380	0.09	0.95	0.1859	7 (18)
Cochrane Pond	4	brook trout	21	0.1990	0.10	0.31	0.0558	0 (0)
		ouananiche	21	0.2138	0.08	0.84	0.1565	1 (5)
Bay Bull's Big Pond	5	brook trout	20	0.1275	0.06	0.21	0.0513	0 (0)
Mobile First Pond	6	brook trout	20	0.1650	0.09	0.24	0.0450	0 (0)
		ouananiche	21	0.4014	0.05	1.02	0.3088	6 (29)
Mobile Big Pond	7	brook trout	27	0.2048	0.12	0.40	0.0642	0 (0)
		ouananiche	23	0.2943	0.07	1.04	0.2614	5 (22)
Mount Carmel Pond	8	brook trout	30	0.6270	0.08	1.81	0.5080	13 (43)
		ouananiche	14	0.3043	0.08	1.39	0.3209	1 (7)
Trinity Pond	9	ouananiche	17	0.2965	0.16	0.41	0.0734	0 (0)
		arctic charr	30	0.2153	0.12	0.36	0.0592	0 (0)
Sandy Lake	10	brook trout	27	0.3337	0.07	1.05	0.3238	6 (22)
		ouananiche	22	0.4277	0.10	1.09	0.3059	9 (41)
Hind's Lake	11	brook trout	14	0.1364	0.09	0.29	0.0518	0 (0)
		ouananiche	31	0.2016	0.05	0.57	0.1154	1 (3)
Red Indian Lake	12	brook trout	17	0.1147	0.08	0.22	0.0354	0 (0)
		ouananiche	18	0.1661	0.05	0.48	0.1009	0 (0)
Joe Dennis Pond	13	brook trout	26	0.1885	0.08	0.38	0.0831	0 (0)
Great Burnt Lake	14	brook trout	28	0.4504	0.19	1.18	0.2864	7 (25)
		ouananiche	26	0.8908	0.14	3.07	0.7481	15 (58)
Cold Spring Pond	15	brook trout	48	0..2715	0.09	1.60	0.2187	3 (6)

		ouananiche	31	0.6045	0.23	2.17	0.4797	12 (39)
		arctic charr	10	0.3340	0.25	0.39	0.0523	0 (0)
Long Pond	16	brook trout	33	0.1637	0.06	1.04	0.1637	1 (3)
		ouananiche	83	0.7270	0.16	2.17	0.3950	55 (66)
		arctic charr	22	0.3323	0.25	0.52	0.0670	1 (5)
Cat Arm	17	brook trout	58	0.2597	0.10	0.52	0.0942	1 (2)
		arctic charr	114	0.6294	0.17	1.05	0.1757	87 (76)
	1982	arctic charr	27	0.1530	0.10	0.26	0.0443	0 (0)
	1982	brook trout	29	0.1090	0.05	0.34	0.0642	0 (0)
Granite Lake	18	brook trout	10	0.2530	0.12	0.42	0.1082	0 (0)
		ouananiche	36	0.5950	0.11	2.00	0.3920	19 (53)
		arctic charr	25	0.3830	0.21	0.64	0.1147	2 (8)
Meelpaeg Lake	19	ouananiche	34	0.7570	0.21	1.39	0.3177	25 (74)
		arctic charr	27	0.2500	0.16	0.45	0.0806	0 (0)

3.4.5. Correlations:

3.4.5.1. Ouananiche:

Linear relation of $\log_{10}$ (Hg) to $\log_{10}$ (FL) was confirmed by visual examination. Slopes determined from regression analysis of $\log_{10}$ (Hg) versus $\log_{10}$ (FL) (Table 3.6.), were found to be negatively correlated with the age of reservoir ($r = -0.695$, $p = 0.00282$; Figure 3.2.) and the conductivity ($r = -0.674$, $p = 0.00586$). Area flooded was not a significant correlate in either case despite an obvious trend in slope with area flooded (Figure 3.3). The slope of $\log_{10}$ (Hg) versus $\log_{10}$ (FL) did not correlate significantly with any of the other physical, biological, and chemical characteristics of the study sites in this investigation (Table 3.7).

Regression was used to estimate the relationship between slope and AR and conductivity ($r^2 = 0.551$, $n = 15$; Equation 1). Area flooded was included in the analysis but did not increase the explained variance and therefore, was not retained in the equation.

$$\text{Slope}_{\text{log Hg vs log FL}} = 2.14 - (0.00970\ \text{AR}) - (0.0212\ \text{Conduct}) \qquad \text{Equation 1.}$$

Table 3.6.: Slopes and y-intercepts with 95% confidence interval (CI) determined from the regression of $\log_{10}$ (Hg) vs $\log_{10}$ (FL) for ouananiche collected at each of the study sites. Information on the fork lengths obtained at each site is also given.

Site	Slope (95% CI)	y-intercept (95% CI)	Fork Length (cm)				
			Mean	Std. Dev.	Min.	Max.	n
1	1.52 (0.154)	-2.78 (0.233)	33.84	10.71	16.0	48.4	26
2	2.30 (0.311)	-3.96 (0.447)	27.86	6.49	17.4	42.7	26
3	1.83 (0.330)	-3.14 (0.469)	13.50	7.50	7.50	55.2	40
4	0.50 (0.212)	-1.37 (0.272)	20.57	8.85	6.10	39.4	21
6	1.10 (0.132)	-1.94 (0.172)	22.47	11.74	4.40	39.7	21
7	1.16 (0.168)	-2.16 (0.223)	22.67	10.61	6.10	44.3	23
8	0.92 (0.228)	-1.73 (0.278)	17.81	7.35	5.70	32.0	14
9	0.55 (0.231)	-1.37 (0.345)	31.91	7.33	20.7	41.5	17
10	1.29 (0.156)	-2.27 (0.220)	27.19	11.40	8.70	46.5	22
11	1.43 (0.222)	-2.65 (0.296)	22.08	5.96	11.1	36.5	31
12	0.92 (0.229)	-2.03 (0.297)	22.54	11.55	9.80	50.3	19
14	2.32 (0.195)	-3.67 (0.294)	33.15	9.28	16.5	50.5	26
15	1.67 (0.196)	-2.59 (0.270)	24.64	8.83	15.0	49.1	32
16	2.22 (0.164)	-3.39 (0.236)	28.06	5.96	16.1	49.1	83
18	1.81 (0.317)	-2.91 (0.456)	28.0	6.78	14.0	40.3	36
19	0.58 (0.358)	-1.05 (0.546)	34.0	6.30	12.4	42.6	34
Totals			26.6	8.67	5.70	55.2	470

Table 3.7.: Pearson correlation coefficients for the slope of the regression of $\log_{10}$ (Hg) vs $\log_{10}$ (FL) for ouananiche with various characteristics of the study sites.

Variable	Correlation Coefficient	p-Value	n
Age	-0.695	0.00282	16
Area Flooded	0.0577	0.838	15
Secchi Depth	0.0651	0.825	14
pH	-0.330	0.229	15
Conductivity	-0.674	0.00586	15
Nitrates	0.253	0.363	15
TP	0.492	0.508	4
DO	0.343	0.211	15
Chlorophyll	0.00788	0.978	15
POC	-0.344	0.209	15
C:N	0.191	0.496	15
% Area Flooded	-0.267	0.325	15

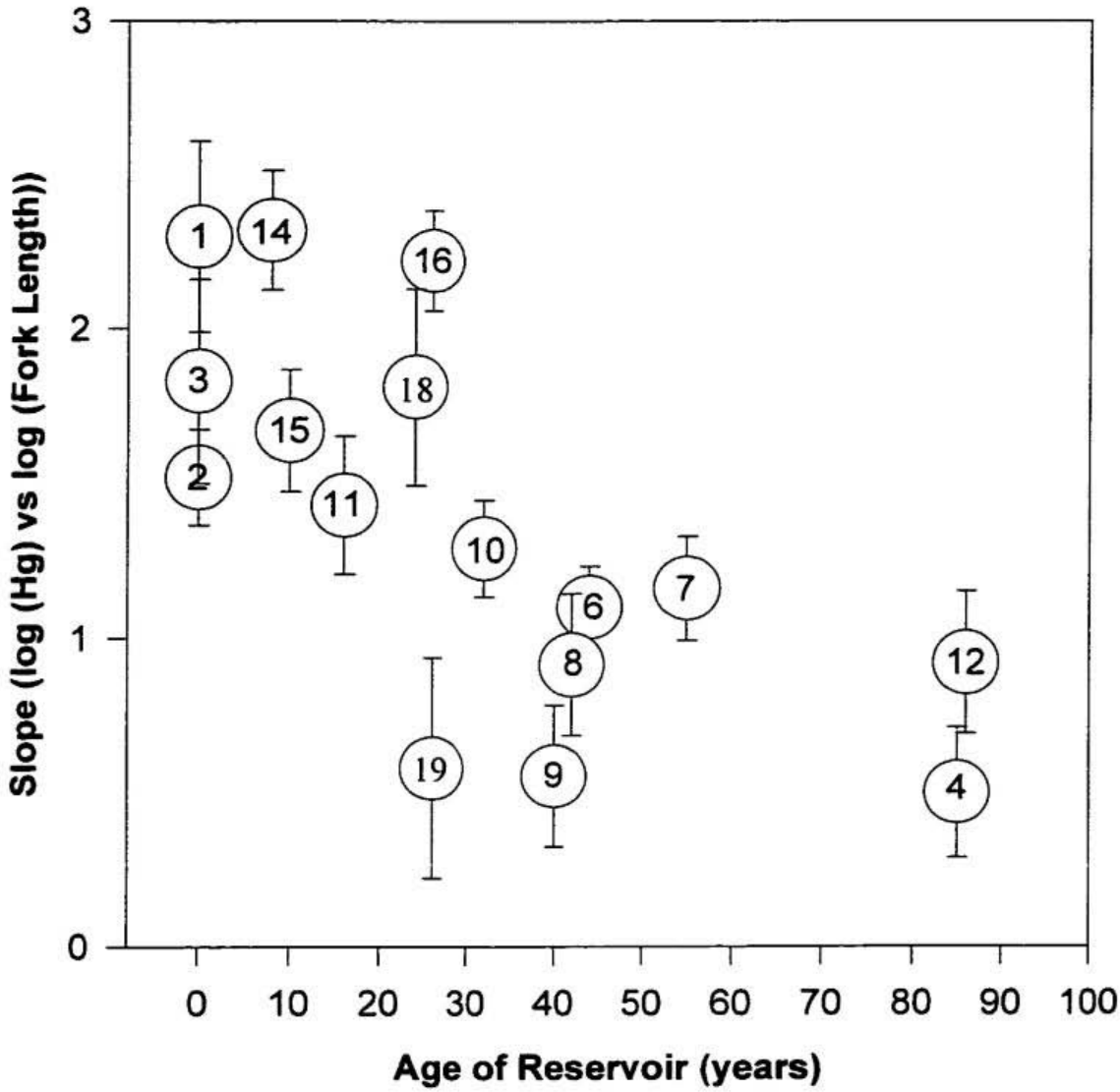

Figure 3.2.: Plot of Slope $_{\log_{10}(\text{Hg (ppm)}) \text{ vs } \log_{10}(\text{FL (cm)})}$ vs AR for ouananiche. Numbers within symbols represent the sample site number: vertical bars represent the 95% CI. Sites 1,2, and 3 are control sites.

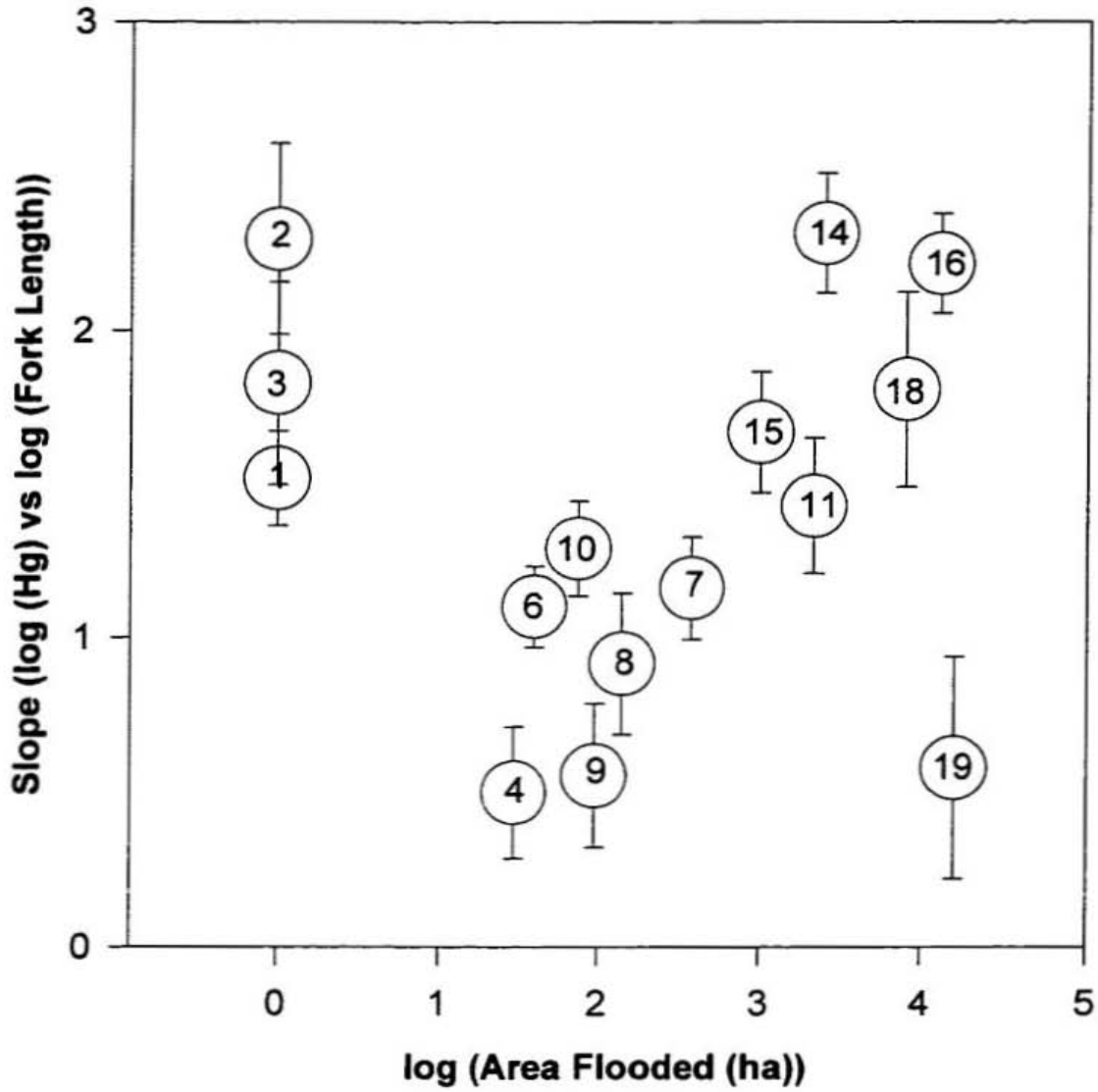

Figure 3.3.: Plot of Slope $_{(\log_{10}(\text{Hg (ppm)}) \text{ vs } \log_{10}(\text{FL (cm)})}$ vs $\log_{10}$ (AF) for ouananiche collected from each of the sampling sites. Numbers within symbols represent the site number; vertical bars represent the 95% CI. Sites 1,2, and 3 are controls.

3.4.5.2. Brook Trout:

Slopes of $\log_{10}$ (Hg) versus $\log_{10}$ (FL) for brook trout were negatively correlated with the reservoir age ($r = -0.5168$, $p = 0.0281$, Figure 3.4.) and the conductivity ($r = -0.6165$, $p = 0.0110$). Regression incorporating AR and conductivity was used to estimate the relationship with slope ($r^2 = 0.401$, $n = 16$; Equation 2). Slopes appeared to increase with area flooded (Figure 3.5), but the area flooded did not increase the explained variance of the regression model; therefore it was not included.

$$\text{Slope}_{\log \text{Hg vs } \log \text{FL}} = 1.75 - (0.00542\ \text{AR}) - (0.0249\ \text{Conduct}) \qquad \text{Equation 2.}$$

Based on Figures 3.4. and 3.5., the reservoirs appeared to fall into two groups - a group with lower slopes ranging from -0.08 to 0.93 and a group with higher slopes ranging from 1.18 to 2.21 (Table 3.8). Within groups, slopes from reservoir fish appeared to decrease as the reservoir aged and to increase as the impoundment area increased (Figure 3.5). Slopes within these two groups were not significantly correlated with any of the other variables investigated (Table 3.9).

Table 3.8.: Slopes and y-intercepts with 95% confidence interval (CI) determined from the regression of $\log_{10}$ (Hg) vs $\log_{10}$ (FL) for brook trout collected at each of the study sites. Information on the fork lengths obtained at each site is also given.

Site	Slope (95% CI)	y-intercept (95% CI)	Fork Length (cm)				
			Mean	Std. Dev.	Min.	Max.	n
1	0.44 (0.0986)	-1.37 (0.444)	17.43	7.40	6.0	34.4	26
2	1.58 (0.271)	-2.96 (0.348)	20.09	7.49	11.5	44.3	20
3	0.91 (0.115)	-1.75 (0.128)	13.5	7.50	7.50	55.2	40
4	-0.08 (0.268)	-0.62 (0.324)	16.59	4.05	10.2	26.9	21
5	0.07 (0.229)	-1.00 (0.259)	13.94	4.67	5.2	19.7	20
6	0.10 (0.181)	-0.91(0.207)	14.28	4.56	5.50	21.7	20
7	0.52 (0.12)	-1.33 (0.145)	16.60	5.33	6.10	25.3	27
8	1.66 (0.201)	-2.39 (0.249)	18.20	7.58	6.0	35.1	30
10	1.73 (0.329)	-2.87 (0.427)	20.79	8.06	11.8	45.1	27
11	0.67 (0.262)	-1.74 (0.318)	19.21	5.12	12.1	26.1	14
12	0.42 (0.268)	-1.46 (0.255)	15.66	3.95	11.6	24.3	17
13	1.18 (0.208)	-2.20 (0.255)	17.32	4.47	11.6	23.9	26
14	1.81 (0.632)	-2.94 (0.883)	25.16	3.70	17.9	34.2	28
15	1.88 (0.369)	-3.04 (0.474)	19.49	3.51	15.9	35.1	48
16	0.57 (0.555)	-1.43 (0.732)	21.05	3.17	16.8	26.4	33
17	0.83 (0.190)	-1.68 (0.245)	20.21	3.76	8.40	27.5	103
18	2.21 (1.20)	-3.45 (1.53)	18.8	1.99	15.6	21.5	10
Totals			18.5	5.80	5.2	55.2	539

Table 3.9.: Pearson correlation coefficients for various characteristics of the study sites with the slope of the regression of $\log_{10}$ (Hg) vs $\log_{10}$(FL) for brook trout.

Variable	Correlation Coefficient	p-Value	n
Age	-0.5382	0.0258	18
Area Flooded	0.128	0.636	17
Secchi Depth	-0.418	0.121	15
pH	-0.477	0.062	16
Conductivity	-0.617	0.011	16
Nitrates	0.265	0.321	16
TP	0.426	0.574	4
DO	-0.136	0.616	16
Chlorophyll	-0.141	0.602	16
POC	0.232	0.387	16
C:N	0.0138	0.960	16
% Area Flooded	-0.434	0.159	16

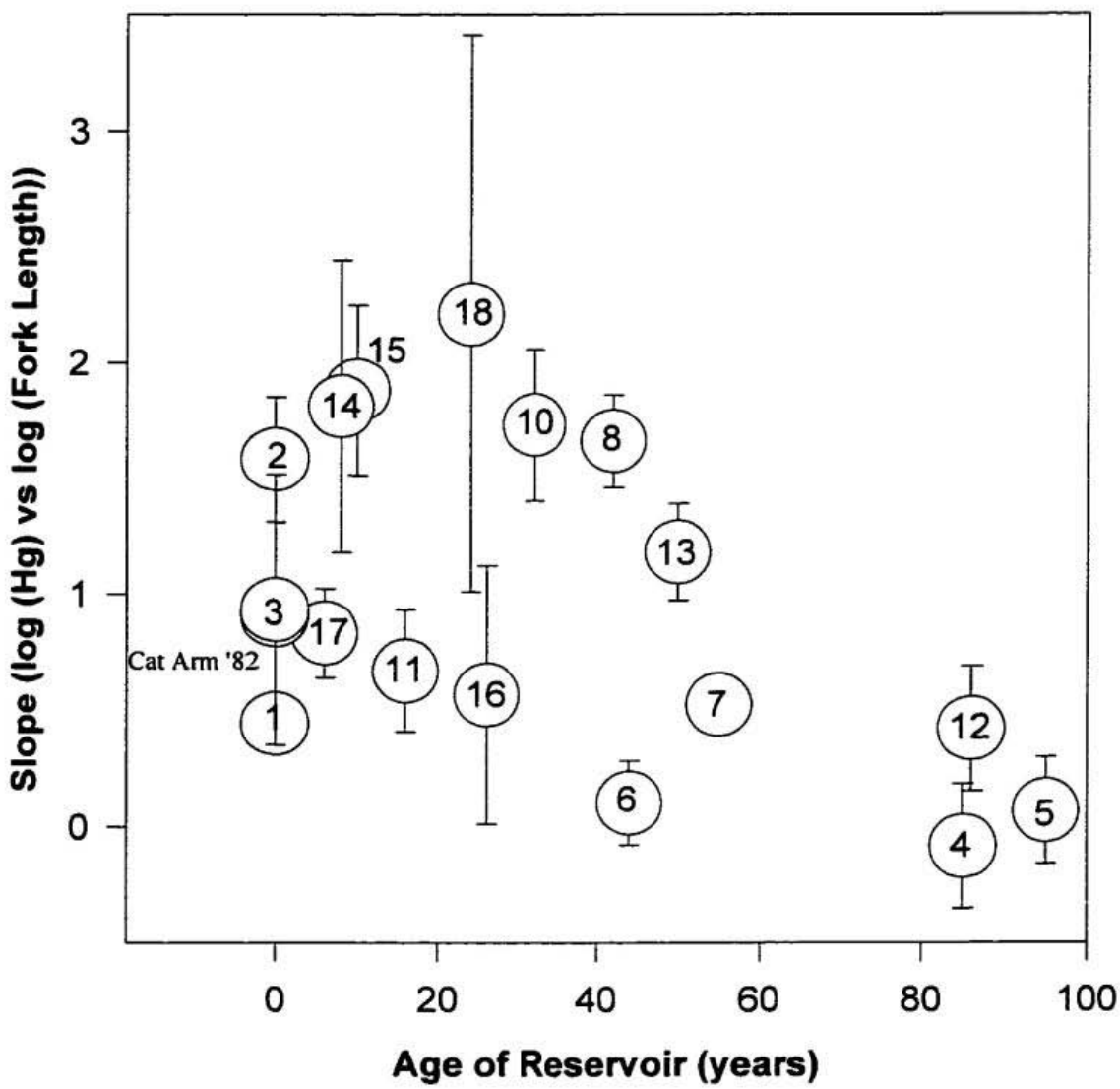

Figure 3.4.: Plot of Slope$_{(\log_{10}(\text{Hg (ppm)}) \text{ vs } \log_{10}(\text{FL (cm)})}$ vs AR for brook trout. Numbers within symbols represent the sample site number; vertical bars represent the 95% CI. Sites 1,2, and 3 are controls.

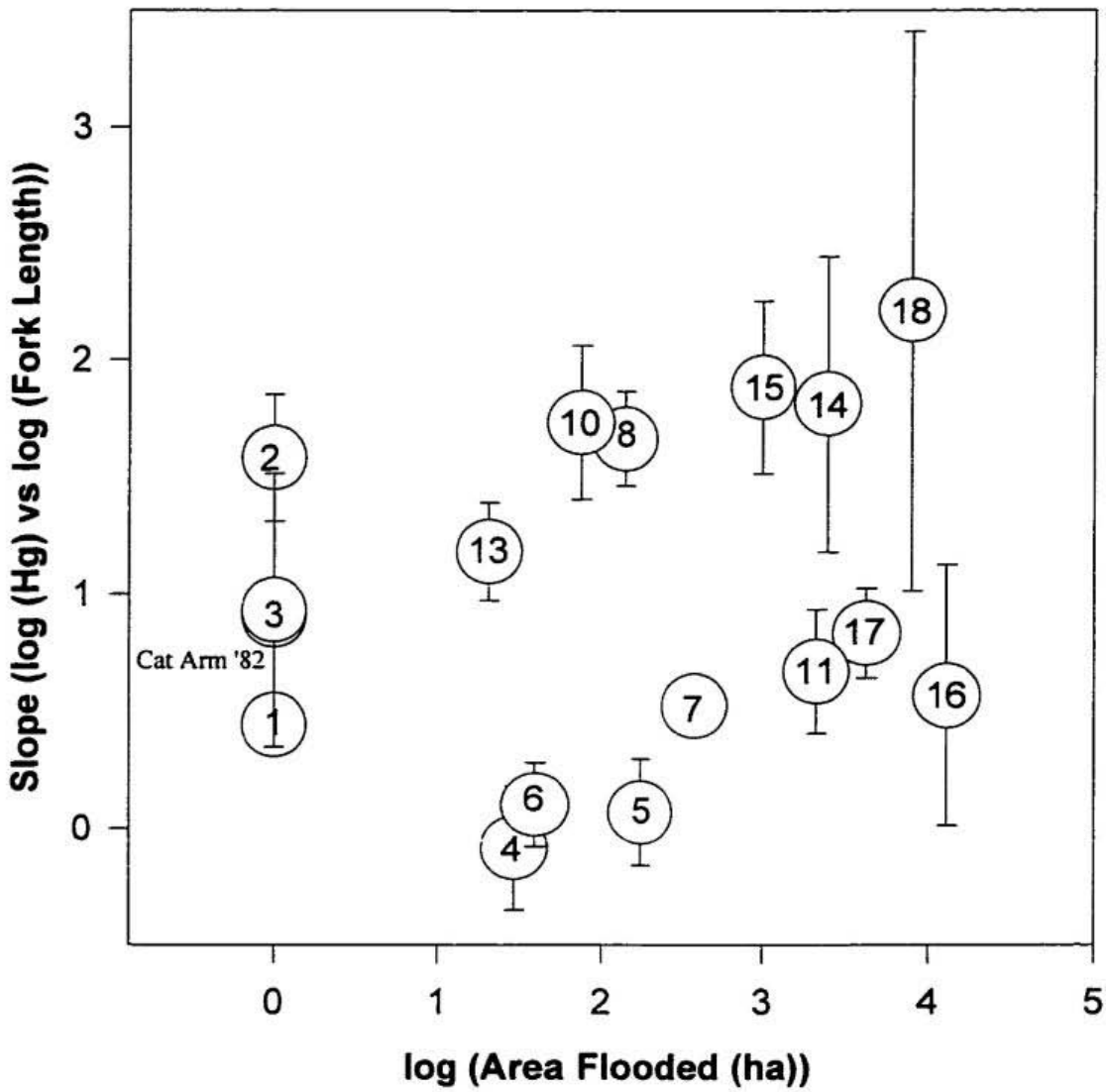

Figure 3.5.: Plot of $Slope_{(\log_{10}(Hg\ (ppm))\ vs\ \log_{10}(FL\ (cms))}$ vs $\log_{10}$ (AF) for brook trout. Numbers within symbols represent the sample site number; vertical bars represent the 95% CI; Sites 1,2, and 3 are controls.

3.4.5.3. Arctic Charr:

Arctic charr were caught in only seven reservoirs. Of the 6 reservoirs included in the analyses, the slopes ranged from 0.140 to 0.998 (Table 3.10). Pearson correlation analysis indicated that the reservoir slopes from the regressions were only significantly related to the % Area Flooded ($r = 0.9122$, $p = 0.0308$, Table 3.11). However, reservoir slopes did appear to decline with reservoir age (Figure 3.6.) and increase with area flooded (Figure 3.7). Linear regression incorporating % Area Flooded for each reservoir was used to estimate the relationship ($r^2 = 0.832$, $n = 5$; Equation 3; Figure 3.8).

$$\text{Slope}_{\log Hg \text{ vs } \log FL} = -0.165 + (0.0137 (\% \text{ Area Flooded})) \qquad \text{Equation 3.}$$

Table 3.10.: Slopes and y-intercepts with 95% confidence interval (CI) determined from the regression of $\log_{10}$ (Hg) vs $\log_{10}$ (FL) for arctic charr collected at each of the study sites. Information on the fork lengths obtained at each site is also given.

Site	Slope (95% CI)	y-intercept (95% CI)	Fork Length (cm)				
			Mean	Std. Dev.	Min.	Max.	n
Cat Arm '82	2.10 (0.501)	-3.25 (0.576)	14.1	1.19	12.5	16.6	27
9	0.14 (0.17)	-0.86 (0.210)	17.43	4.86	8.5	26.0	30
15	0.142 (1.49)	-0.321 (1.83)	16.82	0.63	15.1	17.3	10
16	0.90 (0.416)	-1.62 (0.522)	17.95	1.72	15.1	21.6	22
17	0.998 (0.170)	-1.53 (0.225)	21.12	2.75	11.2	33.0	143
18	0.477 (0.355)	-1.05 (0.355)	19.5	3.35	12.9	24.6	25
19	0.919 (0.146)	-1.71 (0.174)	15.8	3.98	10.3	21.8	27
Totals			19.0	3.88	8.5	33.0	284

Table 3.11.: Pearson correlation coefficients for the slope of the regression of $\log_{10}$ (Hg) vs $\log_{10}$ (FL) for arctic charr with various characteristics of the study sites.

Variable	Correlation Coefficient	p-Value	n
Age	-0.561	0.190	7
Area Flooded	0.0197	0.9666	7
Secchi Depth	0.0227	0.9659	6
pH	-0.420	0.407	6
Conductivity	-0.564	0.244	6
Nitrates	0.454	0.366	6
TP	-----[1]	-----[1]	2
DO	0.424	0.402	6
Chlorophyll	-0.391	0.444	6
POC	-0.286	0.583	6
C:N	0.687	0.132	6
% Area Flooded	0.912	0.0308	5

[1] Insufficient number to determine correlation.

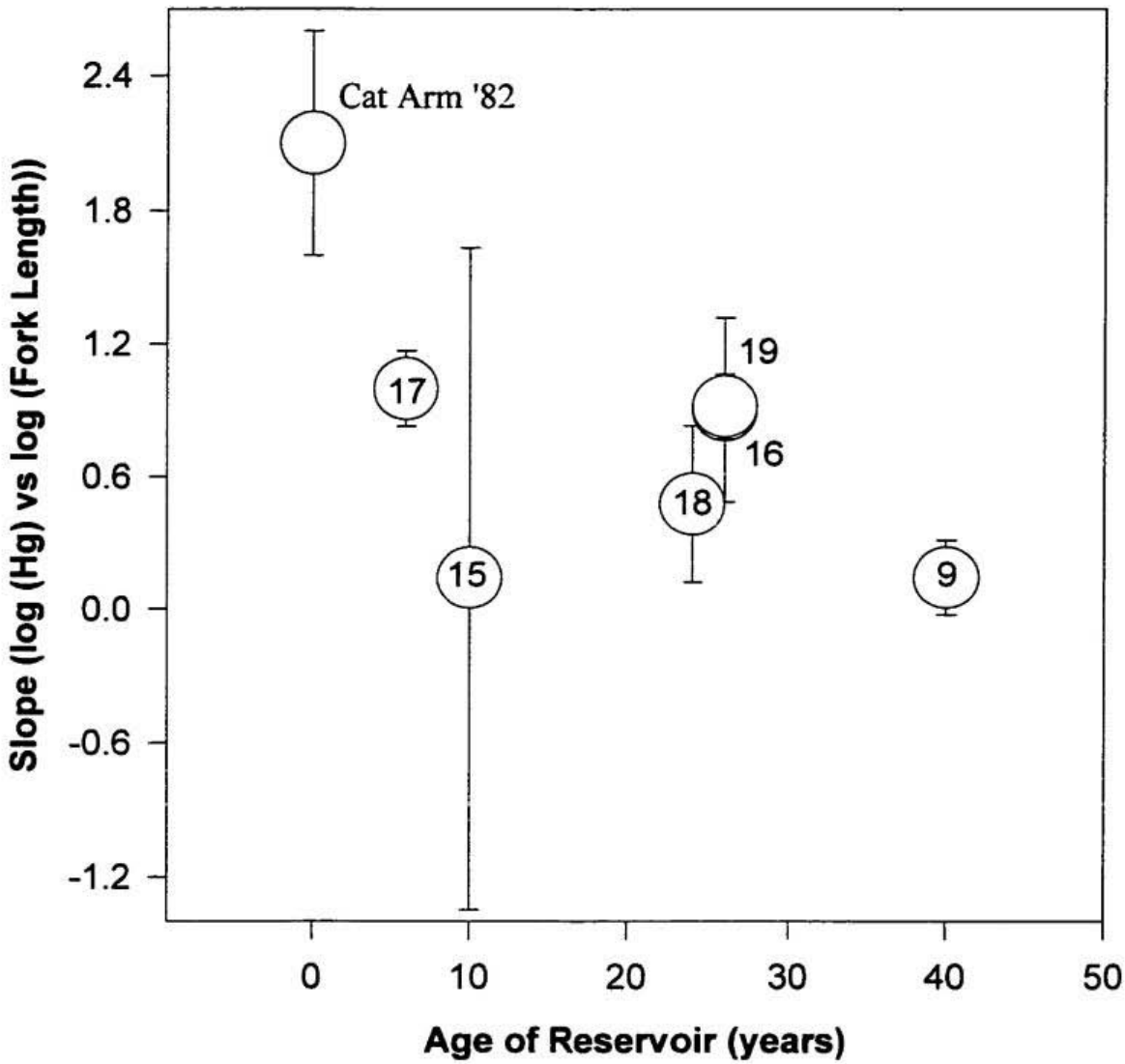

Figure 3.6.: Plot of Slope$_{(\log_{10}(\text{Hg (ppm)}) \text{ vs } \log_{10}(\text{FL (cm)})}$ vs AR for arctic charr. Numbers within symbols represent the sample site number; vertical bars represent the 95% CI.

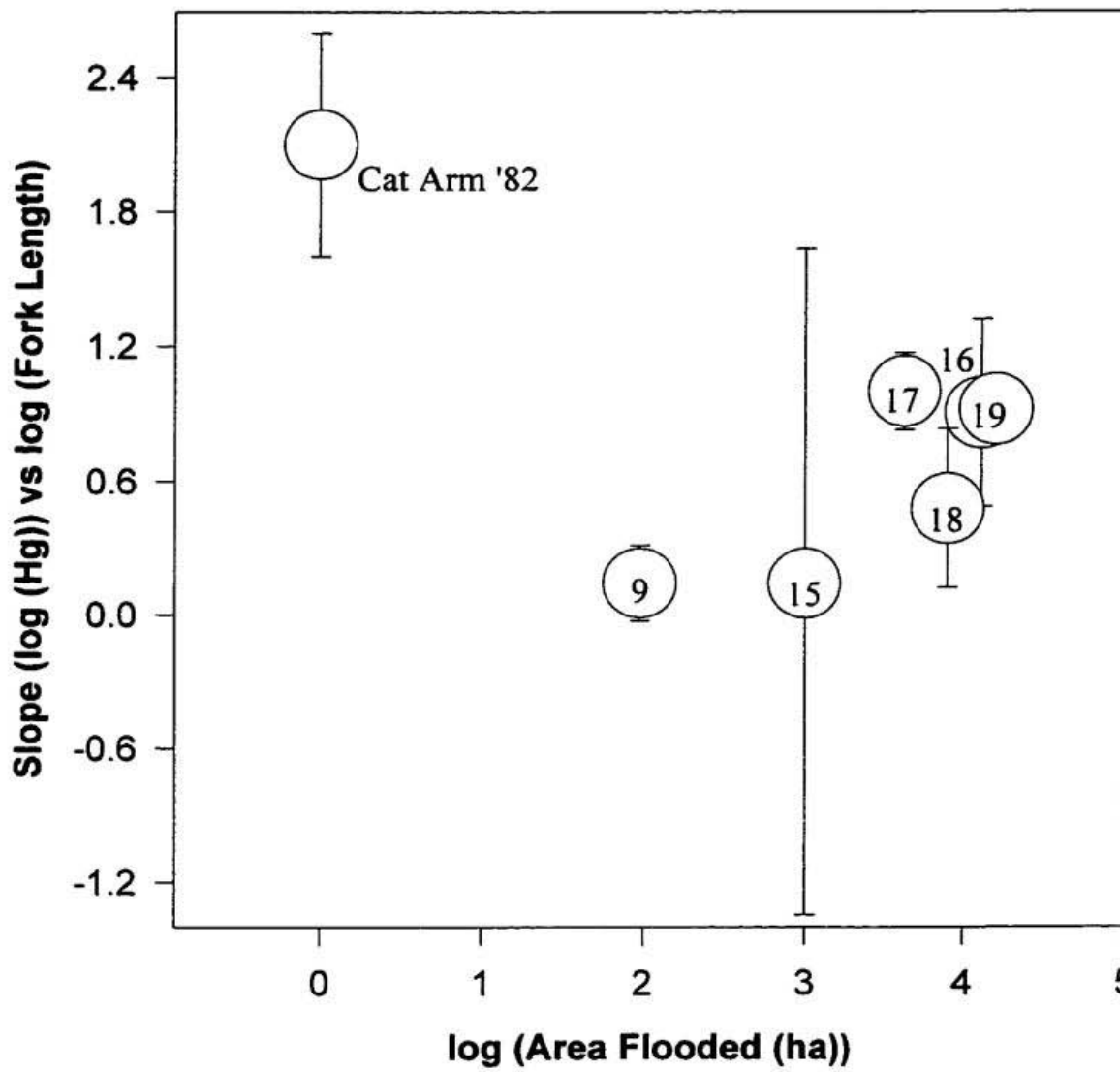

Figure 3.7.: Plot of $Slope_{\log_{10}(Hg\ (ppm))\ vs\ \log_{10}(FL\ (cm))}$ vs $\log_{10}$ (AF) for arctic charr. Numbers within symbols represent the sample site number; vertical bars represent the 95% CI.

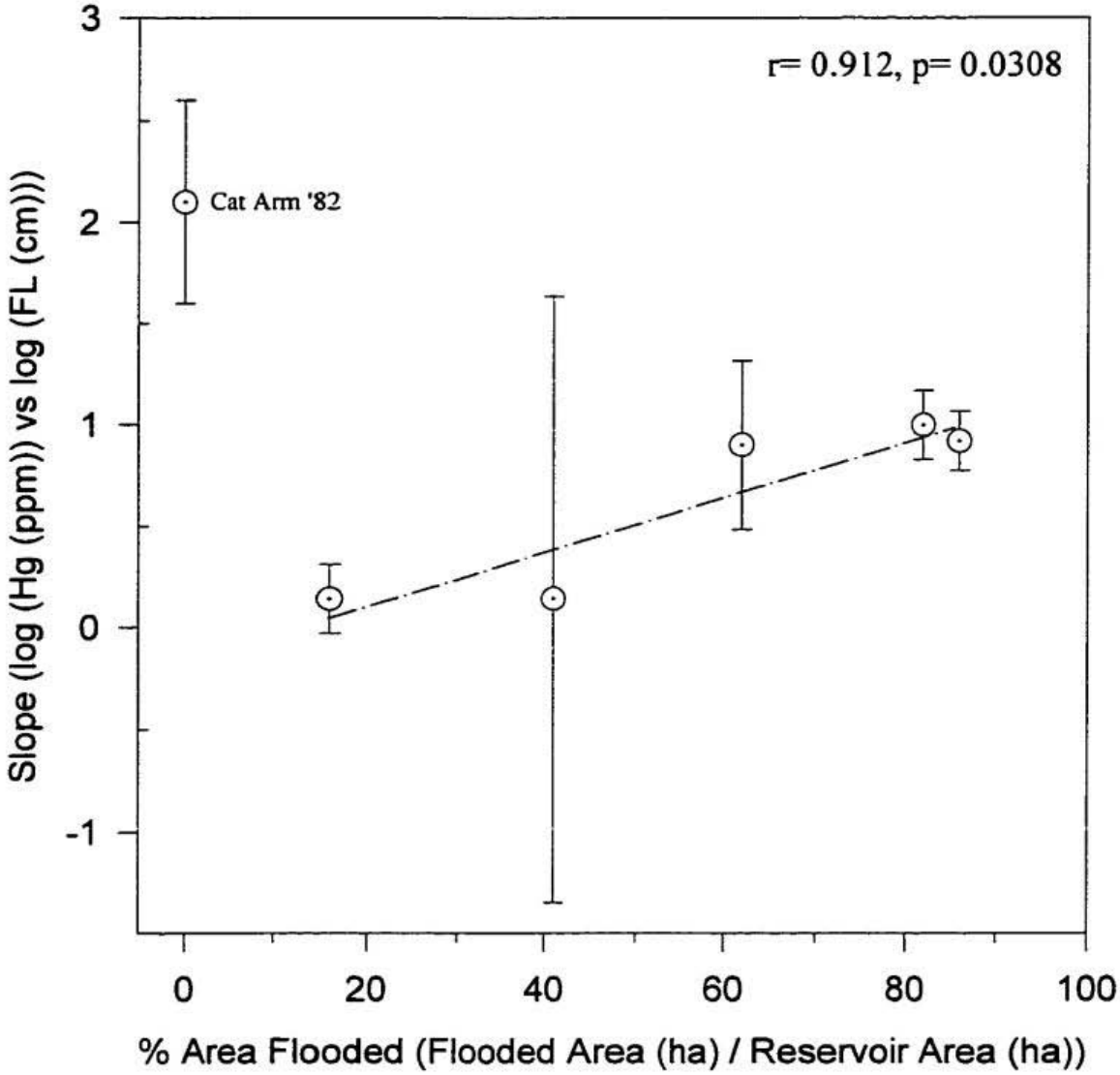

Figure 3.8.: Plot of $Slope_{\log_{10}(Hg\ (ppm))\ vs\ \log_{10}(FL\ (cm))}$ vs % Area Flooded for arctic charr. Vertical bars represent the 95% CI. Broken line represents the least squares regression.

3.4.6. Control Ponds:

There were three control sites and one pre-impounded site utilized in this study as a comparison for the reservoirs (sites 1-3 and Cat Arm '82; Table 3.2.; Figure 3.1). For ouananiche, the slopes observed for the regression of log (Hg) vs log (FL) were highest in Eclipse Pond followed by Middle Gull Pond and Rocky Pond (Table 3.6). For brook trout, the slope was highest in Rocky Pond, followed by Eclipse Pond, Cat Arm '82, and Middle Gull Pond (Table 3.8). Kruskall-Wallis (α=0.05) tests (used due to problems with normality), indicated that there was a significant difference ($H= 35.3$; $p< 0.01$) between mercury levels observed in brook trout collected from the three controls and Cat Arm '82. ANOVA (α=0.05) results indicated that mercury levels in ouananiche collected from the three controls did not differ significantly ($F_{2,91} = 2.24$; $p=0.1129$).

The slopes observed in control ponds and Cat Arm '82 were similar to reservoir slopes. This prevented predictions of return times in reservoirs since it was difficult to determine what represented a pre-impoundment or background slope. For ouananiche and brook trout, the three controls (including Cat Arm '82 in the case of brook trout), demonstrated slopes as high or higher than the reservoirs (Sites 1-3, Figures 3.3.-3.6). Pre-impoundment data for arctic charr from Cat Arm '82 demonstrated the highest slope of all sites investigated (Figures 3.6. and 3.7). However, arctic charr collected in 1982 produced no individuals above the Canadian Safety Limit of 0.5 ppm (Table 3.5). This indicates that both the slope and the absolute amount of mercury per fish of a given size change following impoundment and that while mercury concentration increases, the slope of Hg vs FL actually decreases.

To follow the temporal evolution of the slope of the relationship of log (Hg) vs log (FL) a time series of mercury in fish data from reservoirs operated by and two control ponds sampled by NFLH was used. This database comprised five reservoirs and two control sites (Table 3.2). Results for each year of sampling by reservoir (or control) and by species were regressed ($\log_{10}$ (Hg) vs $\log_{10}$ (FL)) and changes in slopes were examined.

3.4.7. Mercury Time Series:

Cat Arm reservoir (Figure 3.2.) has been monitored since 1982 and it is the only reservoir in NF for which true pre-impoundment data exists. Regression plots of brook trout (Appendix Figures A- 1a. and A- 1b.) and arctic charr (Appendix Figures A- 2a. and A- 2b.) by year indicated that the slope decreased following impoundment and then returned to pre-impoundment levels approximately 5 to 6 years after inundation (Figure 3.9). This suggests that following impoundment there is a rapid increase in mercury in smaller individuals of the population and that there is a lesser change in larger specimens. The higher mercury body burdens in smaller fish caused by flooding lowers the slope from the regression. Pre-impoundment (0 years) fish mercury data from Cat Arm reservoir showed that high slopes exist naturally.

Slopes from Hind's Lake, Long Pond, Cold Spring Pond, and Great Burnt Lake remained relatively high during all sampling years for both species and neither confirmed nor disproved the slope trend seen from Cat Arm. Regression plots for Hind's Lake (Appendix Figures A- 3a., A- 3b., A- 4a., A- 4b., and Figure 3.10.) and Long Pond

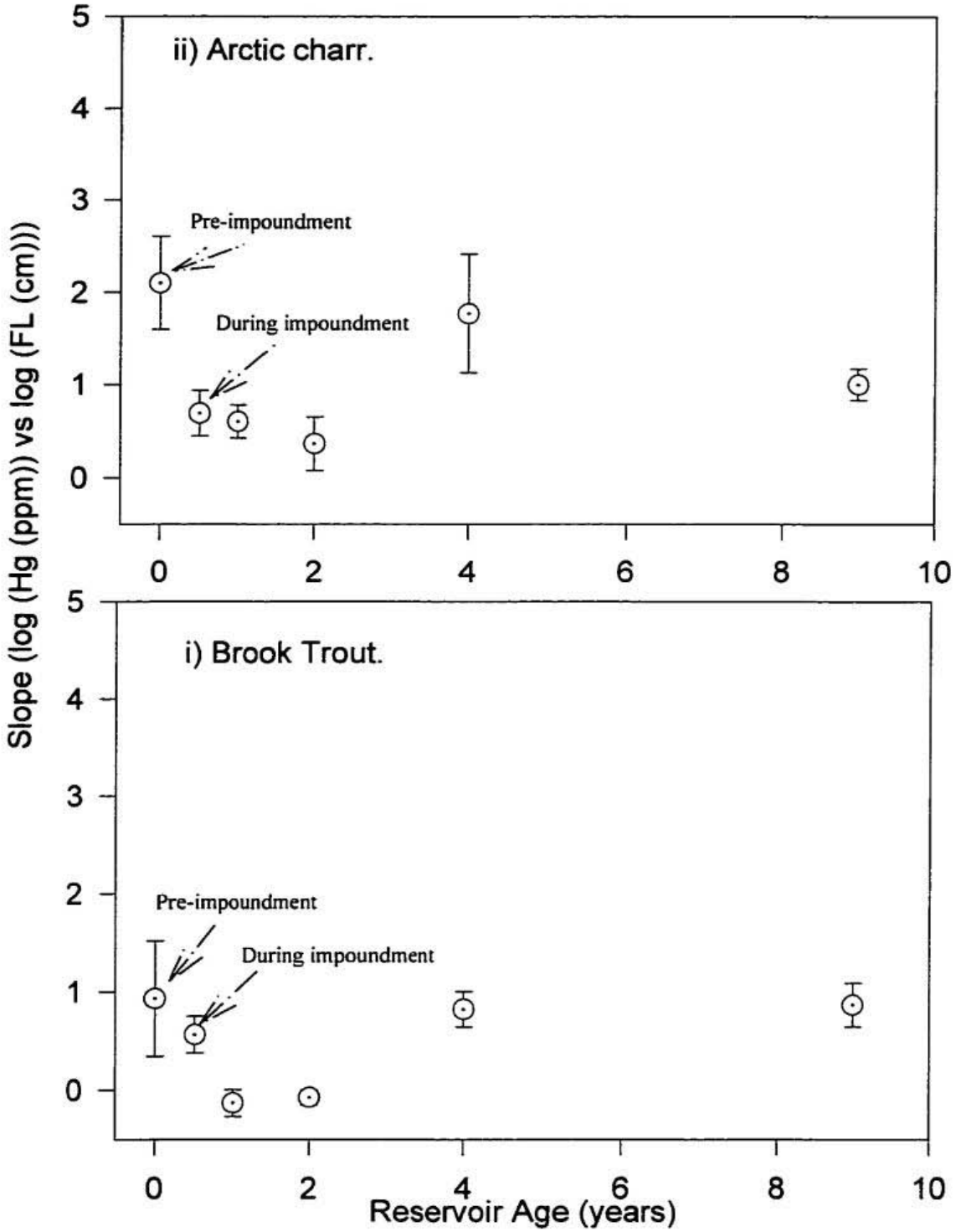

Figure 3.9.: Slopes from the regression of $\log_{10}$ (Hg (ppm)) vs $\log_{10}$ (FL (cm)) for i) brook trout and ii) arctic charr collected from Cat Arm Reservoir plotted against reservoir age. Vertical bars represent the 95% CI.

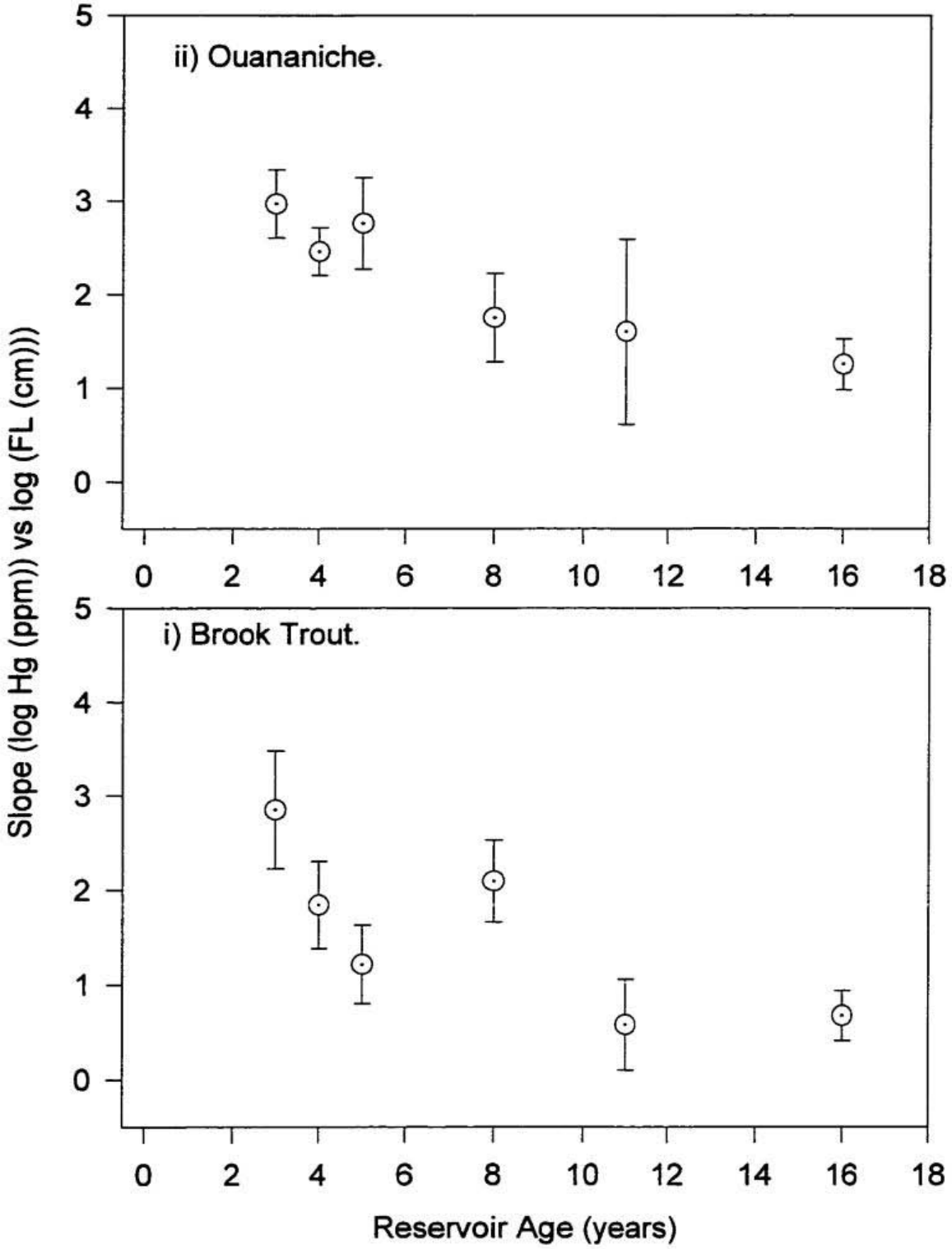

Figure 3.10.: Slopes from the regression of $\log_{10}$ (Hg (ppm)) vs $\log_{10}$ (FL (cm)) for i) brook trout and ii) ouananiche collected from Hind's Lake Reservoir plotted against reservoir age. Vertical bars represent the 95% CI.

(Appendix Figures A- 5a., A- 5b., A- 5c., A- 6a., A- 6b., A- 6c., and Figure 3.11.) showed no evidence of the trend seen in Cat Arm but monitoring in these reservoirs did not begin until three (Hind's Lake) and 16 (Long Pond) years after flooding. As a result, the changes in slope, which appears to occur two to three years following inundation, would have happened prior to the sampling dates. The reservoirs of Cold Spring Pond and Great Burnt Lake are part of the Bay D'Espoir hydroelectric development (see Scruton *et al.*, 1994) which funnels water towards one generating station. Pre-impoundment data exists for Cold Spring Pond and Great Burnt Lake but the data is not true pre-flooding data. Great Burnt Lake and Cold Spring Pond were receiving previously impounded water from upstream as part of the Bay D'Espoir system. Regression plots for brook trout (Appendix Figures A -7a. and A- 7b.) and ouananiche (Appendix Figures A- 8a. and A- 8b.) collected from Cold Spring Pond did not show the trend exhibited in Cat Arm (Figure 3.12). Brook trout from Great Burnt Lake showed no pattern in slopes (Appendix Figure A- 9.) due to the limited numbers of years during which sufficient numbers of specimens were collected (Figure 3.13). Great Burnt Lake ouananiche showed a similar pattern to Cat Arm (Appendix Figures A- 10a. . A- 10b., and Figure 3.13), but it was difficult to test this trend because no 'true' pre-impoundment data exists for Great Burnt Lake.

Eclipse Pond and Rocky Pond were utilized as control ponds and have been monitored since 1982. Regression plots of brook trout (Appendix Figures A- 11a. and A- 11b.) and ouananiche (Appendix Figures A- 12a. and A- 12b.) from Eclipse Pond indicated that slopes remained high during monitoring years with no trend in slope being

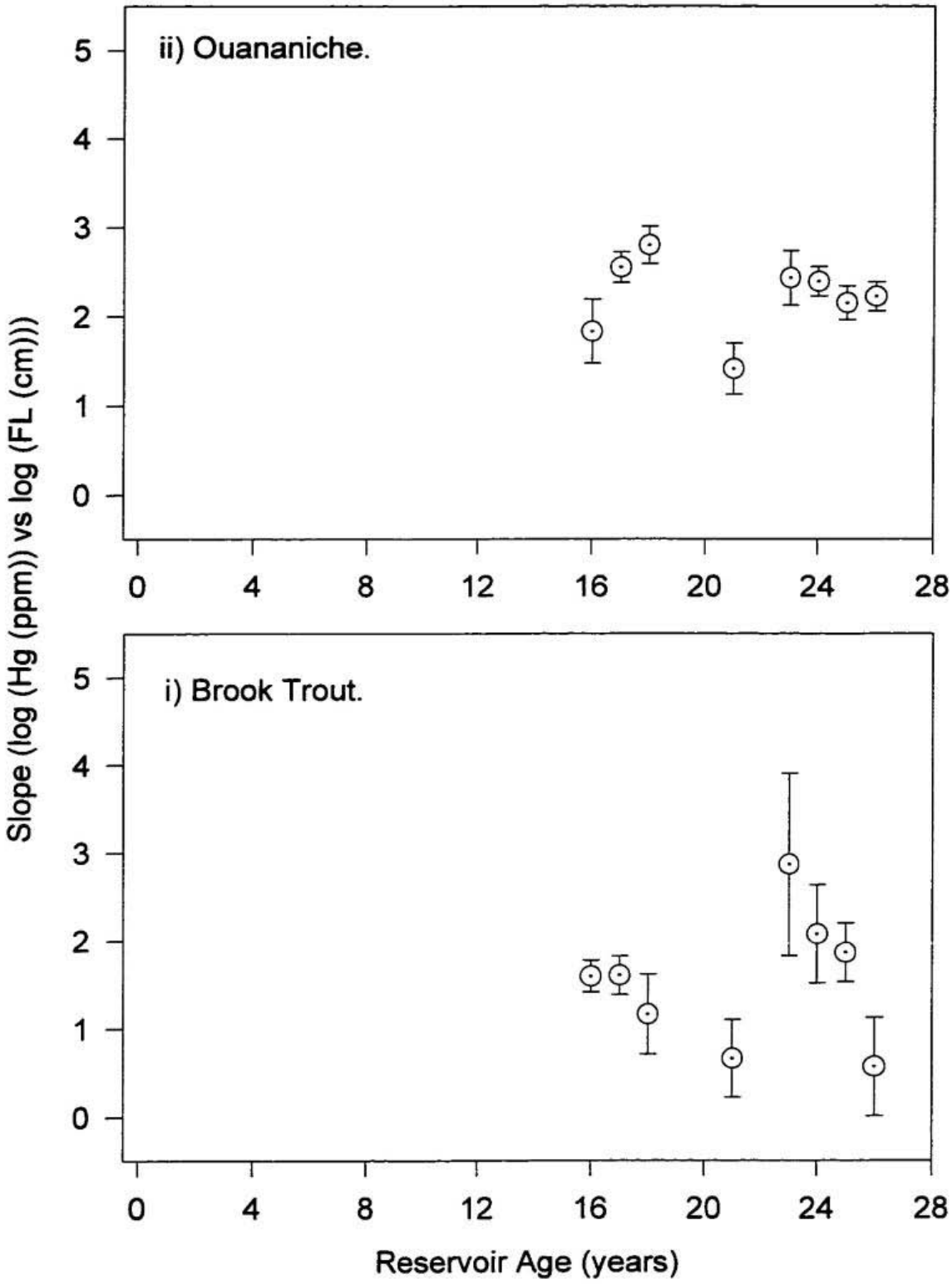

Figure 3.11.: Slopes from the regression of $\log_{10}$ (Hg (ppm) vs $\log_{10}$ (FL (cm)) for i) brook trout and ii) ouananiche collected from Long Pond Reservoir plotted against reservoir age. Vertical bars represent the 95% CI.

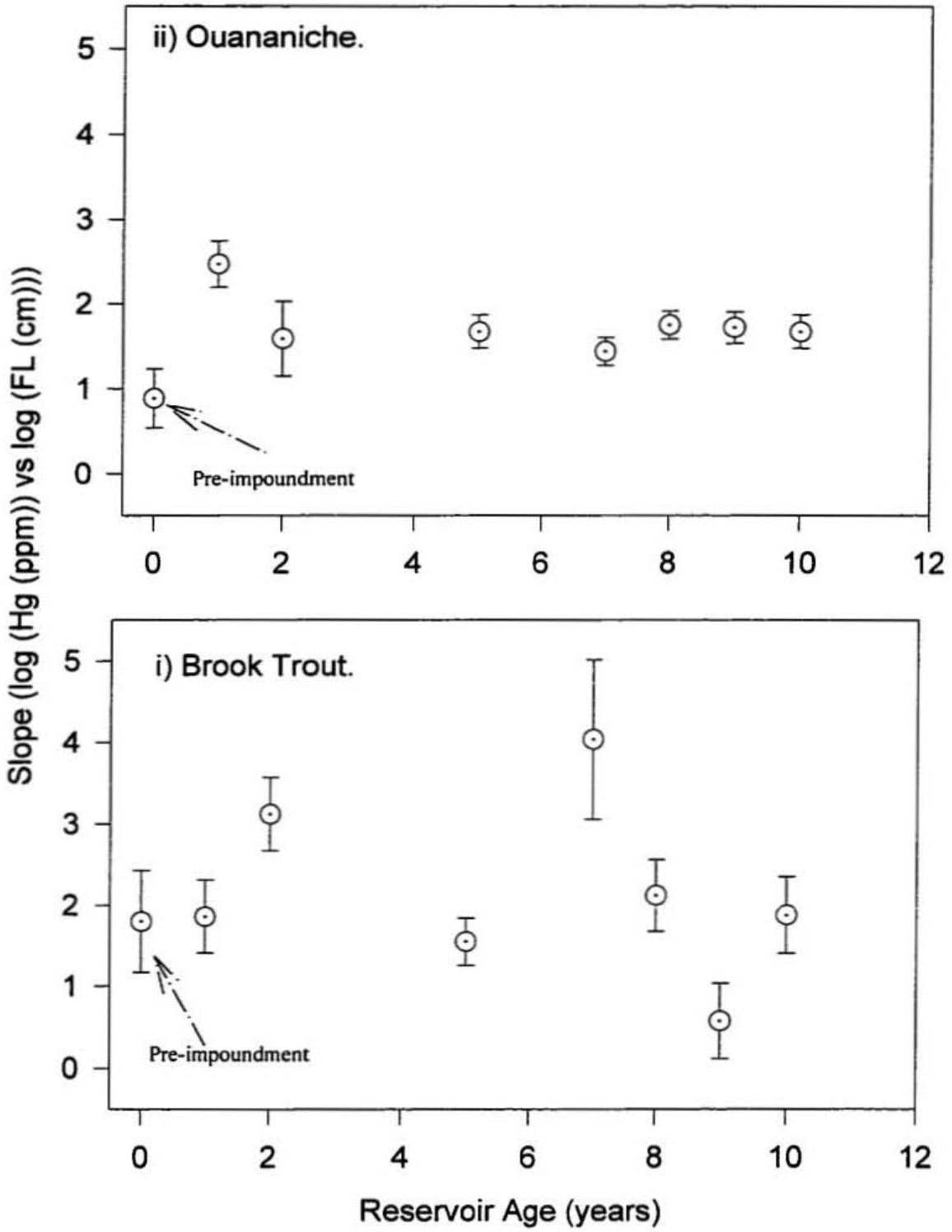

Figure 3.12.: Slopes from the regression of $\log_{10}$ (Hg (ppm)) vs $\log_{10}$ (FL (cm)) for i) brook trout and ii) ouananiche collected from Cold Spring Pond Reservoir plotted against reservoir age. Vertical bars represent the 95% CI.

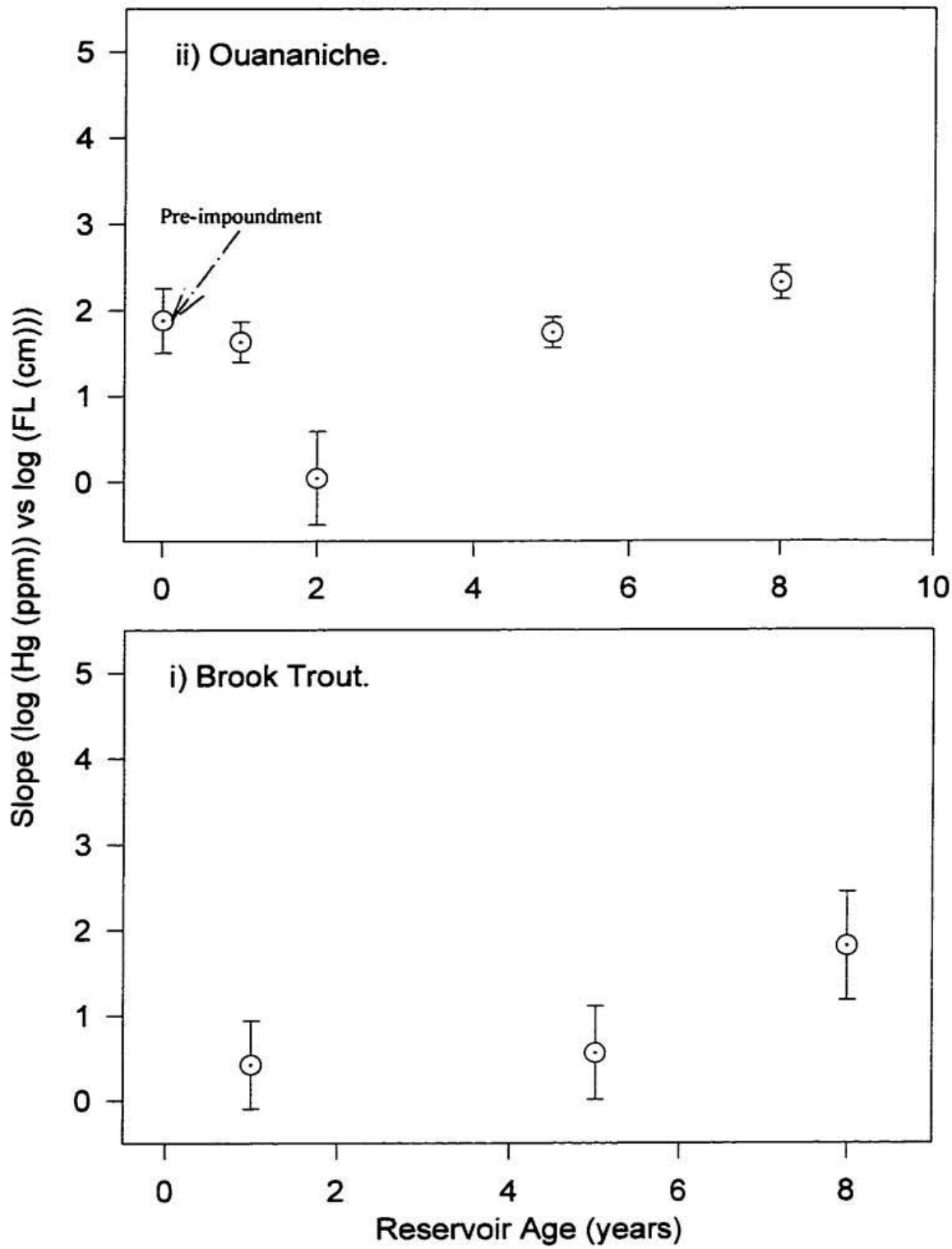

Figure 3.13: Slopes from the regression of $\log_{10}$ (Hg (ppm)) vs $\log_{10}$ (FL (cm)) for i) brook trout and ii) ouananiche collected from Great Burnt Lake Reservoir plotted against reservoir age. Vertical bars represent the 95% CI.

visible (Figure 3.14). Slopes from regression plots for brook trout (Appendix Figure A-13.) and ouananiche (Appendix Figure A- 14.) from Rocky Pond were also high for all of the sampling dates but again, no trend in slopes was visible (Figure 3.15). Results from Eclipse Pond and Rocky Pond indicated that slopes do not change in unimpounded lakes over similar periods of time as that of the reservoirs sampled.

3.4.8. Return Times:

Weighted regression ($\propto$=0.05) was used to make predictions of return times for mercury in ouananiche, brook trout, and arctic charr from reservoirs across the Island. All fork length data for each species were pooled to determine the modal lengths for each species (Table 3.12). A 6 cm size interval was taken about the mode for each species. Individuals that fell within this interval were selected from each year of sampling by reservoir and by species. This controls size variability between sampling sites and years. Fish of a single age were not selected due to high among observer variability in age data. Of those fish that fell within the interval, the mean mercury content was determined and a weight equal to the number used to determine the mean was assigned to that sample point. All data from the time series as well as that from single year sampling in other sites were included by species and by year. Because an exponential model was used, all mercury data was $\log_e$ transformed prior to determination of the mean. Figure 3.16 presents the exponential model of the evolution of mercury in fish following impoundment.

Table 3.12.: Summary statistics for fork lengths (cm) of all species collected as part of the mercury monitoring program.

Statistic	Species		
	Brook Trout	Ouananiche	Arctic Charr
Mean	20.89	28.0389	18.2585
Standard Error	0.1674	0.1825	0.1545
Standard Deviation	6.776	7.6545	3.5742
Size (n)	1638.0	1761	535
Mode	20.0	30	20
Minimum	5.2	4.4	8.5
Maximum	58.6	53.4	33.0

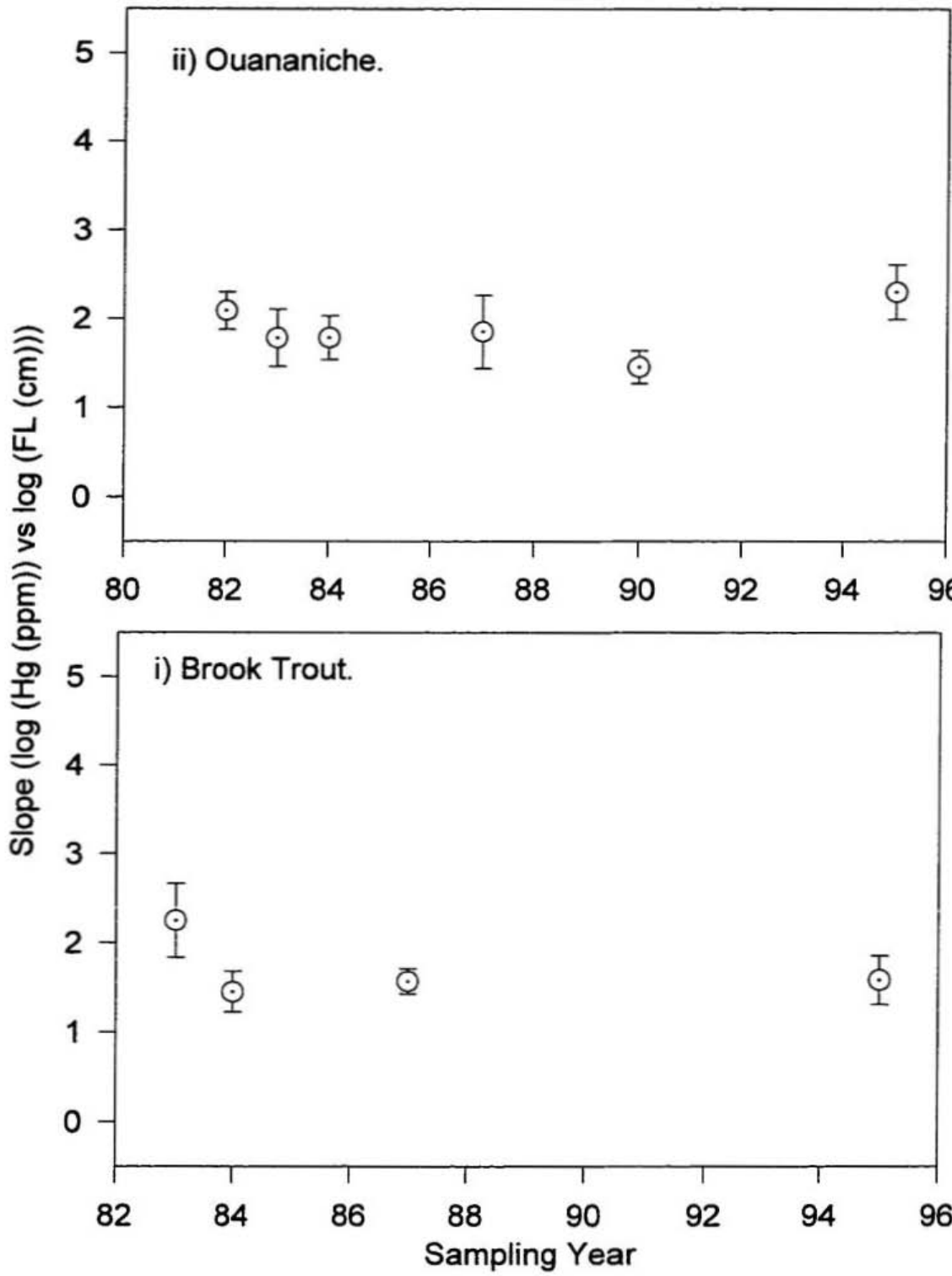

Figure 3.14.: Slopes from the regression of $\log_{10}$ (Hg (ppm)) vs $\log_{10}$ (FL (cm)) for i) brook trout and ii) ouananiche collected from Eclipse Pond control site plotted against sampling year. Vertical bars represent the 95% CI.

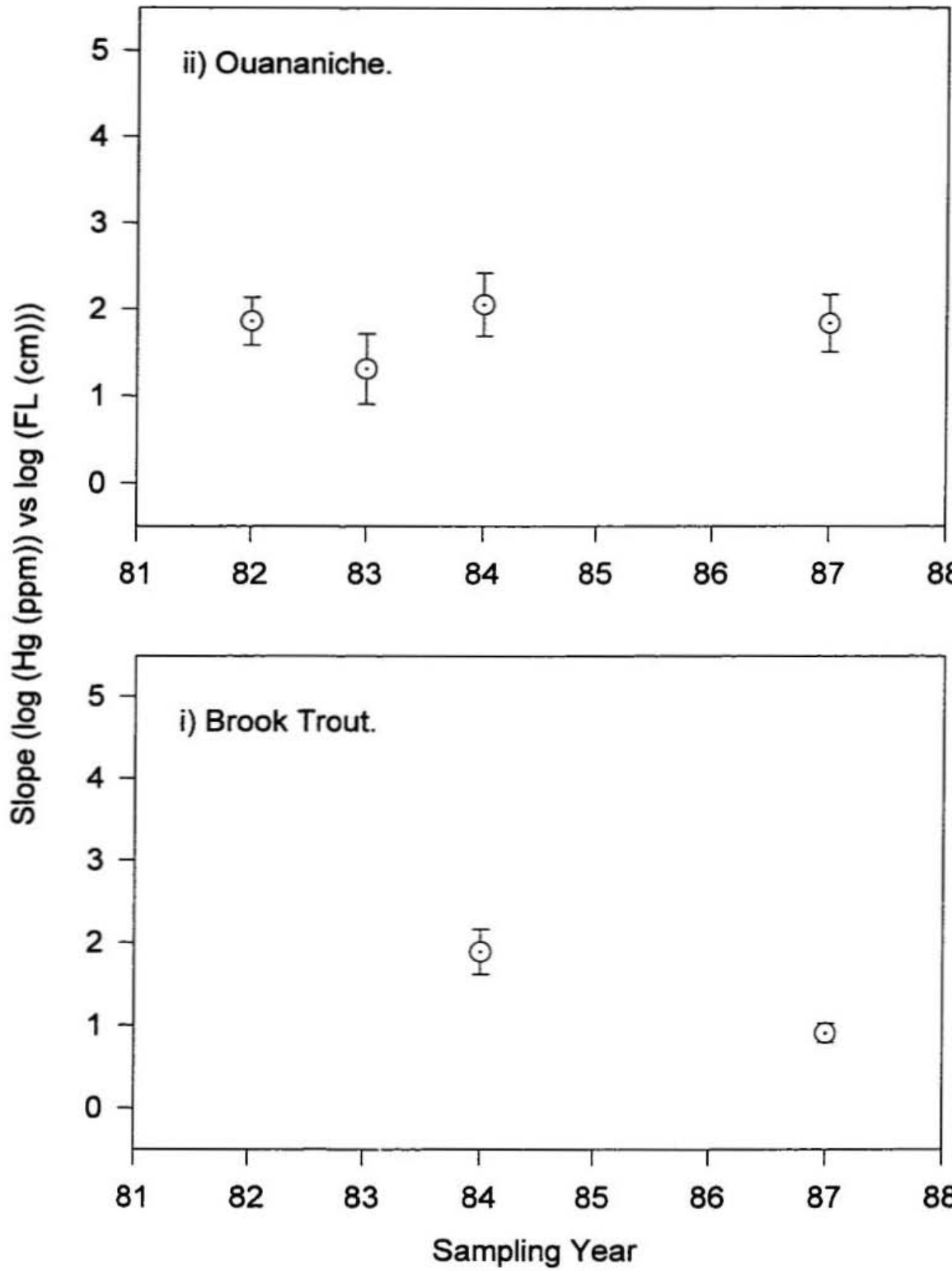

Figure 3.15.: Slopes from the regression of $\log_{10}$ (Hg (ppm)) vs $\log_{10}$ (FL (cm)) for for i) brook trout and ii) ouananiche collected from Rocky Pond control site plotted against sampling year. Vertical bars represent the 95% CI.

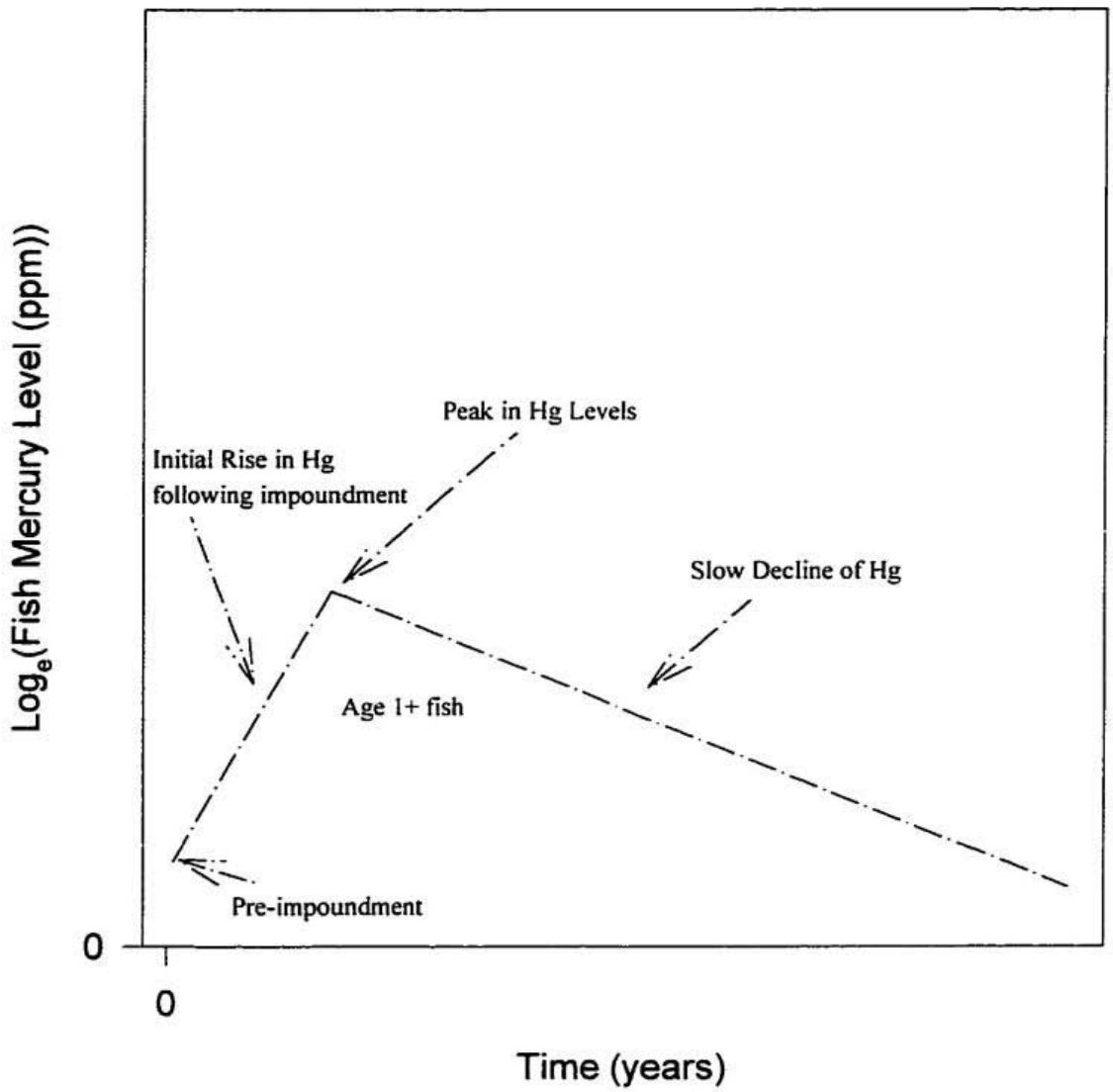

Figure 3.16.: Theoretical model of $\log_e$ (Hg) vs time (years) for age 1+ fish from a newly impounded reservoir.

3.4.8.1. Ouananiche:

The modal length for ouananiche was 30 cm. Therefore, fish were selected from sampling sites in the 27-33 cm range. The mean $\log_e$ Hg values were split by reservoir age prior to regression to determine a rate of increase and a rate of decline. Values were divided at year four following impoundment because this appeared to be the time at which mercury levels peaked.

Weighted regression analysis indicated that the increase in mercury following impoundment to year four was a significant function of reservoir age ($r= 0.672$, $F_{1,17} = 13.94$, $p= 0.002$; Equation 4). Weighted regression analysis indicated that the decrease in mercury after year four was not a significant function of age ($r= 0.290$, $F_{1,29} = 2.66$, $p= 0.113$).

$$Hg = 0.361\ e^{0.304\ AR}\ ;\ AR \leq 4 \text{ years} \quad \text{Equation 4.}$$

Ouananiche in the 27-33 cm size interval can be either insectivorous or piscivorous, and this alteration in trophic status may have obscured the relationship between fork length and mercury content. For this reason, ouananiche were taken in the 15-21 cm range since this smaller size class are more likely to represent a single trophic level.

Mean $\log_e$ values were divided at year two following impoundment because this appeared to be the time at which mercury levels peaked. Weighted regression analysis indicated that the increase observed in ouananiche mercury levels to two years post-impoundment in 15-21 cm fish was a significant function of reservoir age ($r= 0.883$, $F_{1,12}$

= 42.31, p< 0.0001; Equation 5; Figure 3.17). Regression analysis also indicated that mercury decline observed in ouananiche 2 years+ post-impoundment was a significant function of reservoir age (r= 0.592, $F_{1,22}$ = 11.86, p= 0.002; Equation 6; Figure 3.17).

$$Hg = 0.1108\, e^{0.875\,AR}\ ;\ AR \leq 2 \text{ years} \quad \text{Equation 5.}$$

$$Hg = 0.307\, e^{-0.0119\,AR}\ ;\ AR \geq 2 \text{ years} \quad \text{Equation 6.}$$

In Equations 5 and 6, the exponents are instantaneous rates of increase (87.5% $year^{-1}$; Equation 5) or decrease (-1.19% $year^{-1}$; Equation 6).

3.4.8.2. Brook Trout:

The modal length for brook trout was 20 cm. Brook trout were selected in the 17-23 cm size interval. Values were arbitrarily divided at year five following impoundment, the year at which mercury levels peaked. Weighted regression analysis indicated that mercury rise in brook trout to five years post-impoundment was a significant function of reservoir age (r= 0.834, $F_{1,21}$ = 47.82, p< 0.0001; Equation 7, Figure 3.18). Weighted regression analysis indicated that mercury decline in brook trout five years+ post-impoundment was a significant function of reservoir age (r= 0.424, $F_{1,27}$ = 5.93, p= 0.022; Equation 8, Figure 3.18).

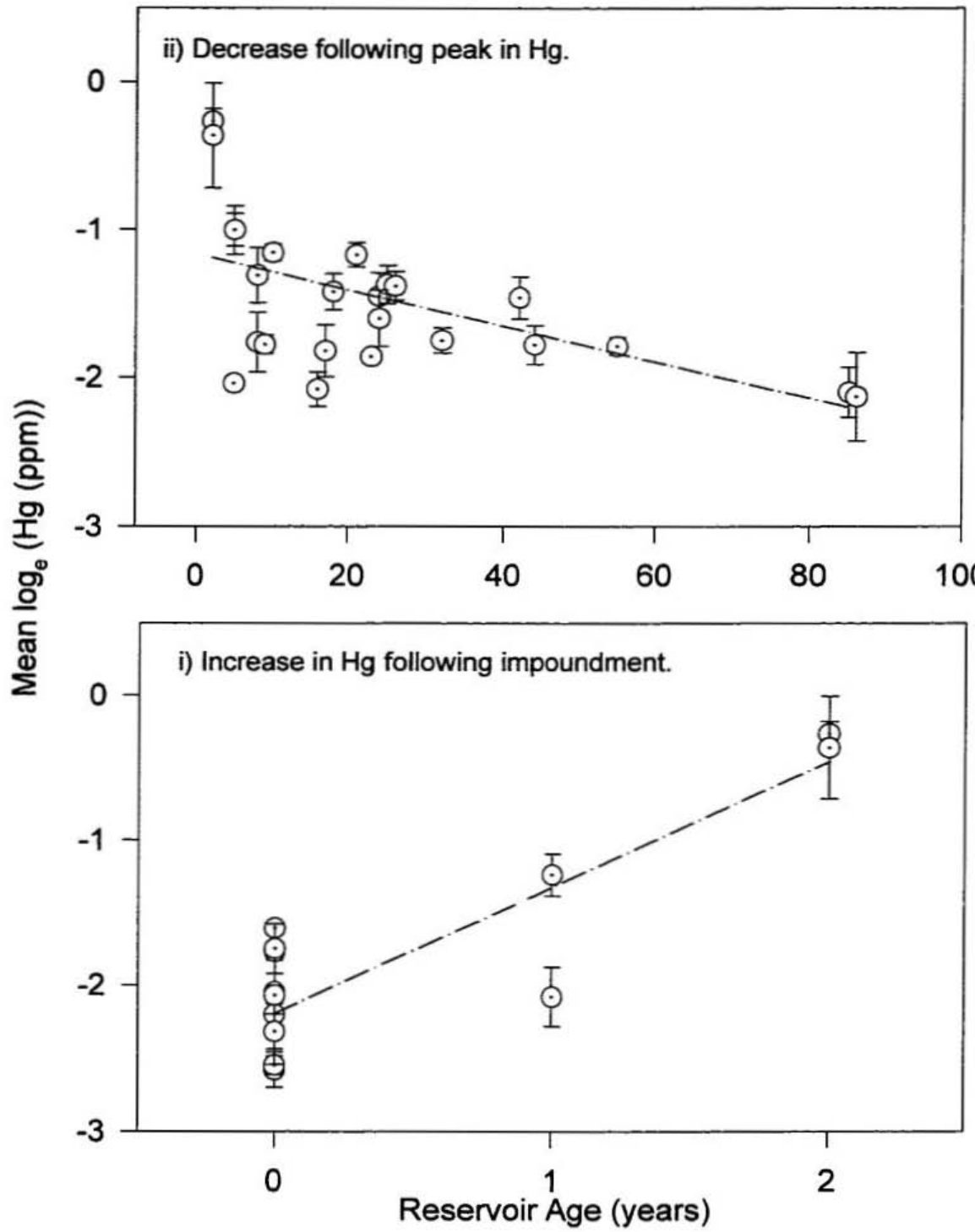

Figure 3.17.: Plots of mean $\log_e$(Hg) vs Reservoir Age for ouananiche collected across NF for i) up to 2 years and ii) 2 years+ following impoundment. Broken lines represent the weighted regressions. Vertical bars represent the 95% CI.

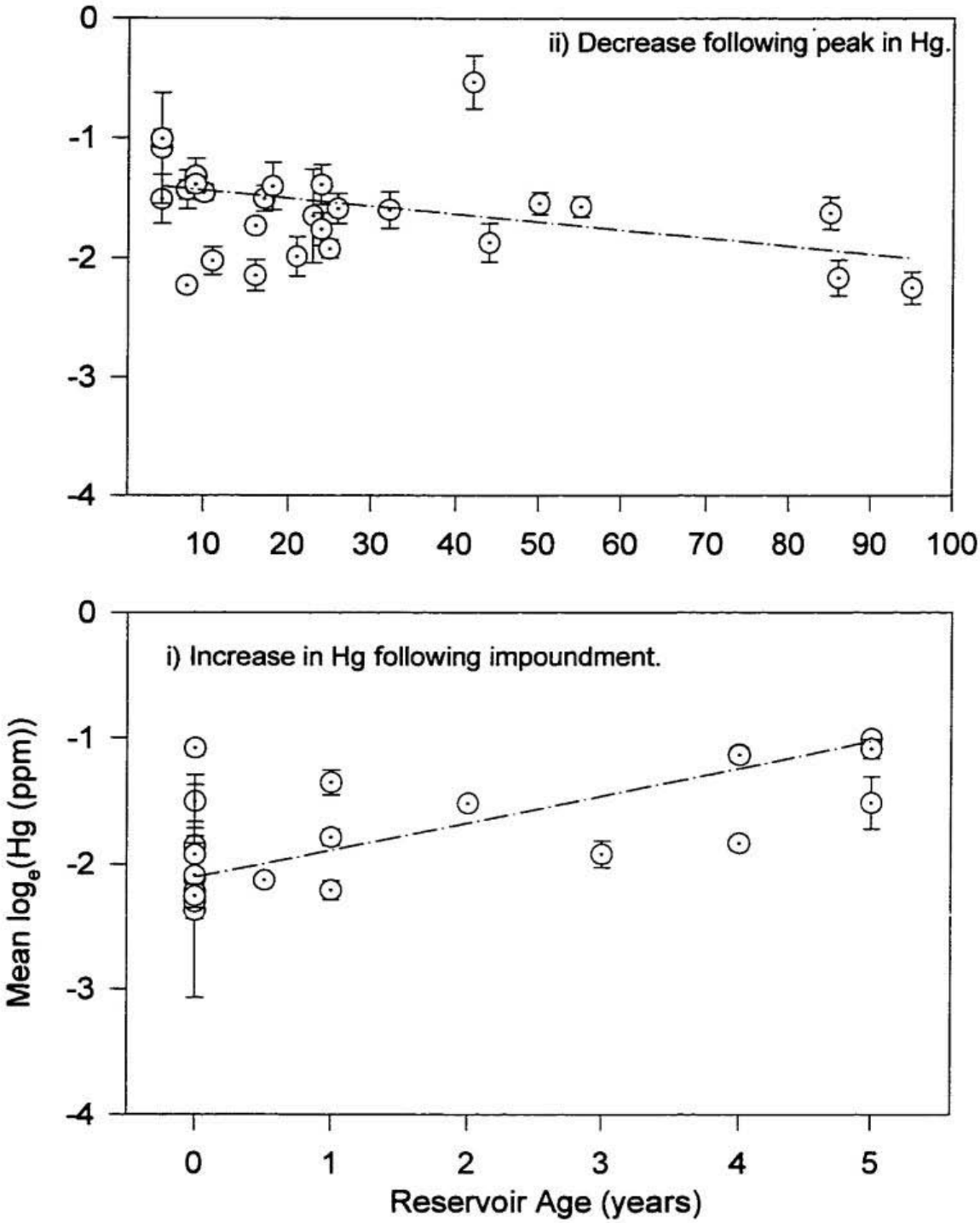

Figure 3.18.: Plots of mean $\log_e$ (Hg) vs Reservoir Age for brook trout collected across NF for i) up to 5 years and ii) 5 years+ following impoundment. Broken lines represent the weighted regressions. Vertical bars represent the 95% CI.

$$Hg = 0.361\ e^{0.337\ AR}\ ;AR \leq 5\ years \qquad \text{Equation 7.}$$

$$Hg = 0.254\ e^{-0.00667\ AR}\ ;AR \geq 5\ years \qquad \text{Equation 8.}$$

3.4.8.3. Arctic Charr:

The modal length for arctic charr was 20 cm. Arctic charr were therefore selected in the 17-23 cm size interval. Values were arbitrarily divided at year four following impoundment, the year at which mercury levels peaked. Weighted regression analysis indicated that mercury rise in arctic charr to four years post-impoundment was a significant function of reservoir age ($r = 0.978$, $F_{1,2} = 44.95$, $p = 0.022$; Equation 9, Figure 3.19). Weighted regression analysis indicated that mercury decline in arctic charr four years+ post-impoundment was a significant function of reservoir age ($r = 0.984$, $F_{1,5} = 149.91$, $p < 0.0001$; Equation 10, Figure 3.19).

$$Hg = 0.257\ e^{0.298\ AR}\ ;AR \leq 4\ years \qquad \text{Equation 9.}$$

$$Hg = 0.842\ e^{-0.0376\ AR}\ ;AR \geq 4\ years \qquad \text{Equation 10.}$$

3.4.8.4. Return Times for Ouananiche, Brook Trout, and Arctic Charr:

In all regressions modelling the decline of mercury in fish, the slope coefficient for age of the reservoir is much less than that of the coefficient for the increase in mercury during the first few years of impoundment. Increase coefficients ranged from 29.8% $year^{-1}$ to 87.5% $year^{-1}$ while decline coefficients ranged from -0.667% $year^{-1}$ to -3.76% $year^{-1}$.

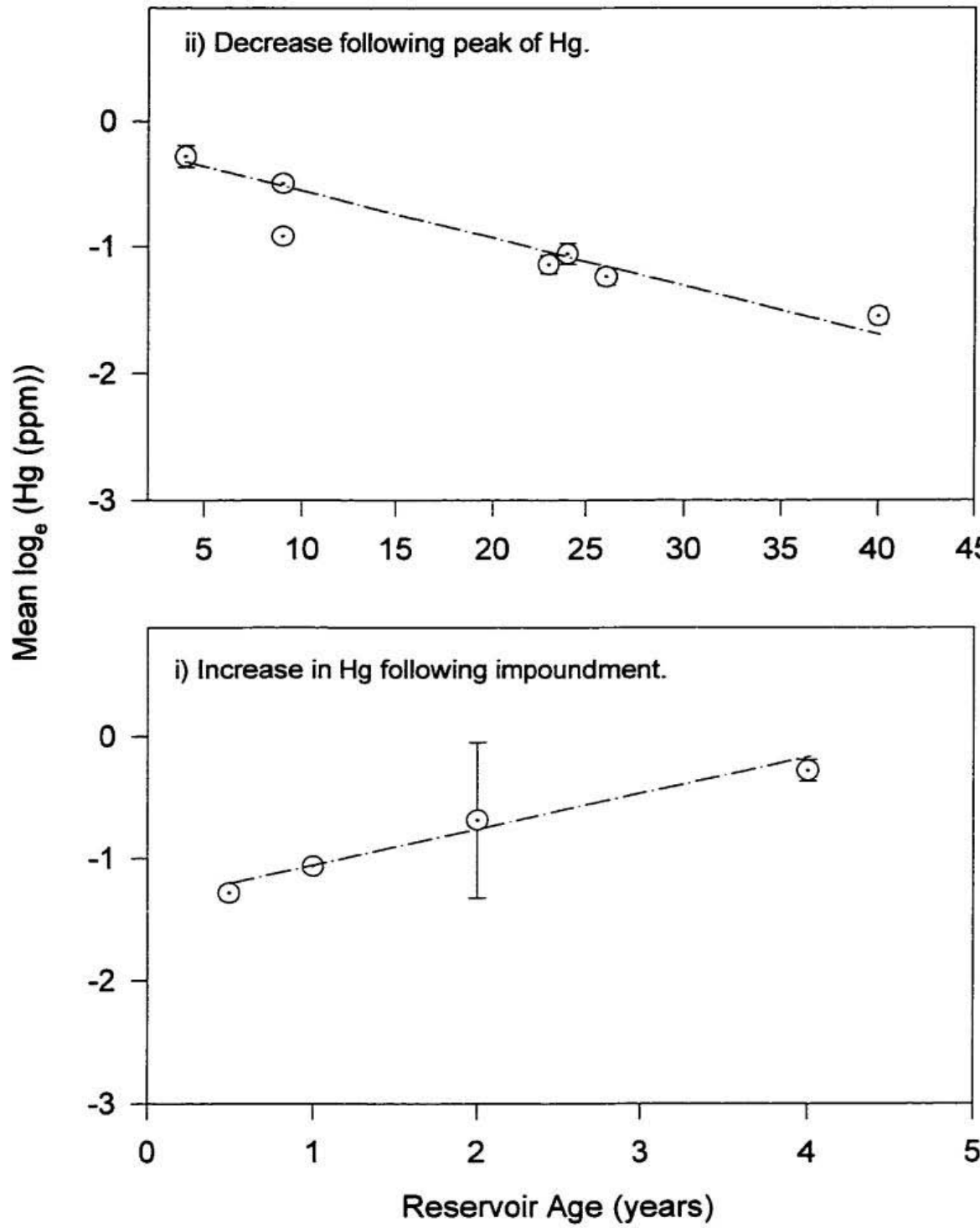

Figure 3.19.: Plots of mean $\log_e$ (Hg) vs Reservoir Age for arctic charr collected across NF for i) up to 4 years and ii) 4 years+ following impoundment. Broken lines represent the weighted regressions. Vertical bars represent the 95% CI.

Equations 5 and 6 indicate that the rate of increase in mercury in ouananiche following impoundment is approximately 0.875 / 0.0119 = 73 times faster than the rate of decline; Equations 7 and 8 indicate that the rate of increase in mercury in brook trout following impoundment is approximately 0.337 / 0.00667 = 50 times faster than the rate of decline; Equations 9 and 10 indicate that rate of increase in mercury in arctic charr following impoundment is approximately 0.298 / 0.0376 = 8 times faster than the rate of decline.

3.5. Discussion:

Traditionally, comparisons between control ponds and impounded sites have been made using either least squares regression or standardized lengths to control for the relation between mercury and fish size. The former approach is valid only if the slopes of both the control and impounded sites are the same (Somers and Jackson, 1993). By using standardized lengths based on regression equations, differences in slopes are ignored. Differences in slope are incorporated into the standardization but are combined with differences in intercept leading to a distortion of the data (Somers and Jackson, 1993). Bivariate relationships between mercury and fish length reduced to single point indices for each reservoir or control pond population result in a significant loss of information (Anderson *et al.*, 1995), because the means and standard deviations are combined with the regression slopes, intercepts, and correlations to estimate a mercury concentration that should be independent of fish length (Somers and Jackson, 1993). Similar size adjusted mercury values may arise even though the original values differed considerably (Somers and Jackson, 1993). In this study, the slope from the relationship between fish mercury levels and fork length was found to change substantially over time and was used to evaluate the temporal variation of mercury in reservoir fish.

3.5.1. Slope Analysis:

Analysis indicated that slopes in impounded and unimpounded sites across NF were a function of the age and the water conductivity for ouananiche and brook trout. Slopes were determined to be a function of only the % Area Flooded for arctic charr.

Reservoir age has been a major factor in modelling mercury decline in fish from reservoirs (Verta *et al.*, 1986; Jackson, 1988; Verdon *et al.*, 1991; Scruton *et al.* 1994; Bodaly *et al.*, 1997). Inundation causes a rapid release of inorganic mercury and organic nutrients from the flooded terrain, both of which can elevate mercury methylation (Wright and Hamilton, 1982). In Finnish reservoirs a high negative correlation was found between reservoir age and mercury content in 1 kg Northern pike (Verta *et al.*, 1986) suggesting that as a reservoir ages, mercury levels in fish decline. Abernathy and Cumbie (1977) found that the lowest concentrations of mercury were found in largemouth bass (*Micropterus salmoides*) from the oldest reservoir while the highest concentrations were found in bass from the youngest reservoir. In this study, reservoir age was negatively correlated with the slope of log (Hg) vs log (FL) in ouananiche and brook trout. This finding supports the notion that elevated mercury in reservoir fish is transitory.

Conductivity was negatively correlated with the slope of log (Hg) vs log (FL) for ouananiche and brook trout collected from reservoirs across NF. Wren and MacCrimmon (1983) found that mercury concentrations in sunfish, *Lepomis gebbosus*, from 16 Precambrian Shield lakes in Ontario, Canada were also significantly correlated ($r = -0.64$) with conductivity. Conductivity reflects water hardness and alkalinity and studies have shown that accumulation of mercury in fish is correlated with decreasing water hardness and alkalinity (Kleinert and Degurse, 1981: Scheider *et al.*, 1981). Wiener *et al.* (1990) found that mercury concentrations in fish of a given species and age are often inversely correlated with lake alkalinity. The correlation of conductivity with the slope of the relationship between mercury content and fork length for ouananiche and brook trout may

therefore reflect an influence on mercury uptake.

Fish accumulate methyl mercury from both food and water. Abernathy and Cumbie (1977) suggested that under some circumstances accumulation from water is more important than ingestion of mercury with food. Phillips and Buhler (1978) found that fingerling rainbow trout (*Salmo gairdneri*) assimilated 10% of methyl mercury that passed across their gills and approximately 68% of all of the methyl mercury they consumed. The direct uptake of waterborne methyl mercury by fish occurs almost entirely across the gills (Olson *et al.*, 1973) and in low conductive lakes, there may be a lack of competing ions in the water column enhancing methyl mercury absorption via this mechanism. Rodgers and Beamish (1983) found that uptake of methyl mercury across the gills was enhanced in waters with low calcium concentrations and low conductivity in NF reservoirs may reflect low calcium concentrations. Uptake of methyl mercury from water may not be the major cause of the correlation between slope and conductivity observed in this study. However, conductivity can augment methyl mercury accumulation in fish by enhancing uptake directly from the water column.

The slope of the relationship between mercury content and fork length in arctic charr was only correlated with the % Area Flooded (Flooded area (ha) / Reservoir Area (ha)). Terrestrial soils flooded as a result of impoundment are a potential source of mercury (Meister *et al.*, 1979; Bodaly *et al.*, 1984), and the % area flooded may therefore reflect the impact of impoundment. The positive correlation between % area flooded and slope suggest that a small % area flooded has less impact on the mercury accumulated in resident fish. The area flooded and % area flooded associated with reservoir creation has

received little attention with respect to the mercury in reservoir fish phenomenon. Because mercury exists as part of the terrestrial environment, the amount of terrain flooded should explain part of the increase seen in reservoir fish following impoundment. Bacterial production can be increased due to the degradation of flooded terrestrial vegetation and probably serves to promote mercury methylation (Bodaly *et al.*, 1984). The influx of organic matter and nutrients from the inundated area can increase the productivity, which has been linked to the amount of mercury methylation (Fagerström and Jernelöv, 1972; Wright and Hamilton, 1982; Gilmour, 1992). The % area flooded can give an indication of the impact that inundation will have on mercury in resident fish populations.

3.5.2. Time Series:

Time series data from Rocky Pond and Eclipse Pond, two non-impounded sites, revealed that mercury can be naturally high in both ouananiche and brook trout. Slopes were not necessarily lower in non-impounded lakes. Moreover, brook trout collected from 11 headwater non-impounded lakes across NF in 1981 demonstrated high variability in the slope of the relationship between $\log_{10}$ (Hg) and $\log_{10}$ (FL). Slopes ranged from -0.83 to 4.31. Four out of the 11 lakes had fish containing mercury above the Canadian Safety Level (French *et al*). Ouananiche collected from 9 lakes during the same survey ranged in slope from -2.62 to 2.44. Six out of the 9 lakes had fish containing mercury above the Canadian Safety Level (French *et al*).

The time series data suggested an explanation for the variability observed in the

slope of the relationship between mercury content and fork length. Results from Cat Arm suggested that following impoundment small fish accumulate mercury rapidly. This decreased the slope from the regression of log (Hg) vs log (FL) because mercury concentrations in larger individuals increased less than those in smaller individuals. Slopes were high before impoundment and were high approximately five to six years after inundation. At that time the increased mercury burden that the small fish had received following impoundment would have passed thru the system as these individuals grew and died. Cat Arm is the only NF reservoir for which pre-impoundment data exists. It is unknown whether this trend is true for other water bodies because pre-impoundment data was not collected for the other time series sites.

Cat Arm was part of a thorough monitoring program into the trophic evolution of a reservoir. Investigations into the plankton community may explain the temporal trend seen in slope. The importance of different foods in the diet of fish following impoundment may influence the transfer of methyl mercury through the food web (Bodaly *et al.*, 1997), but bioaccumulation and biomagnification begin at the lower trophic levels (Cabana *et al.*, 1994) such as that occupied by plankton. The trophic structure in Cat Arm changed dramatically from 4% (of plankton biomass) zooplankton prior to filling in 1983 to 59%, 43%, and 50% zooplankton in 1984, 1985, and 1986, respectively (Copeman and Knoechel, 1988). The plankton community increased substantially for approximately three years until 1986 when the mean biomass was considerably lower (Copeman and Knoechel, 1988). Maximum concentrations of methyl mercury have been found in zooplankton during the early stages of impoundment (Bodaly

et al., 1997) suggesting an explanation of why slopes were lower relatively flat in Cat Arm for the first few years following inundation. Bioaccumulation of Hg released from the flooded area to zooplankton could have resulted in more rapid accumulation in younger smaller fish after impoundment than before impoundment. Plankton is the main vector for mercury into a fish population, and small fish that consumed more food (plankton) higher in mercury relative to pre-impoundment years would accumulate more methyl mercury. The return of slopes to pre-impoundment levels in Cat Arm during the 1988 and 1993 sampling years parallelled the decrease in plankton biomass that began in 1986. In the earlier years following impoundment, there appeared to be more plankton and mercury available to the fish community.

Time series data from Cat Arm indicated that small fish accumulated relatively more mercury than large fish following impoundment. This suggests it is more efficient to monitor these younger fish to establish the impact of inundation on mercury accumulation. Age-0 perch, *Perca flavescens*, have been used to follow mercury uptake rates since their specific respiration and consumption rates are substantially higher than those of older and larger fish and therefore will accumulate mercury at higher rates (Post, 1990), providing a more sensitive system for examining uptake processes (Post *et al.*, 1996). Monitoring younger, smaller fish would require less effort as only fish in a specific size class corresponding to a pre-selected age would be collected each year. Small fish are easier to catch and numbers collected would not have to be great (e.g. 10 fish per year). Use of electro-fishing rather than the usual gill net equipment would also lead to less wastage and less impact upon spawning members of the resident fish populations.

3.5.3. Return Times:

Mercury increase following impoundment was found to be a function of reservoir age for ouananiche, brook trout, and arctic charr. As well, the decline in fish mercury content following the peak in mercury levels for each species was found to be a function of reservoir age. The slope coefficient for the decline of mercury with the age of the reservoir was 8 to 73 times lower than that for the early increase in post-impoundment mercury. This quantifies the notion that mercury levels rise quickly following impoundment and then slowly return to background levels (Johnston *et al.*, 1991).

This study also demonstrated the need for pre-impoundment data to predict return times. The available data indicated that each site may have its own pre-impoundment level, and determination of what comprises a pre-impoundment level for reservoirs for which no pre-impoundment data exists is problematic.

Models indicated that mercury increase in fish following inundation was a function of reservoir age. This will not predict the time mercury levels will peak. A time series for each specific site is needed to determine the return times since it difficult to find a peak mercury level that will apply to all reservoirs. To establish return times for a particular species from a reservoir, a pre-impoundment level would have to be inserted into the model. For example, to determine the return time for brook trout from Cat Arm, the mean pre-impoundment mercury level (0.1049 ppm) for the specific size class would be inserted into the model (Equation 8) describing the rate of decline:

$$Hg = 0.254\ e^{-0.00667\ AR}\ ;AR \geq 5\ years \qquad \text{Equation 8.}$$

$$0.1049 = 0.254\ e^{-0.00667\ AR}$$

$$-0.88433 = -0.00667\ AR$$

$$AR = 132\ years + 5\ years\ (to\ peak)$$

$$AR = 137\ years$$

The return time predicted for brook trout (137 years) is large. However, the model does not take into account the life span of the particular species and because the mercury data was from reservoirs across NF that ranged in age from 9 to 95 years , there is much spatial and temporal variation built into the model.

3.6. Conclusions:

The slope from the regression of $\log_{10}$ (Hg) vs $\log_{10}$ (FL) was found to change with reservoir age. Slopes decreased with the age and the conductivity of the reservoir for ouananiche and brook trout. Slopes were found to increase with only the % Area Flooded for arctic charr. Models incorporating these variables for each species were formulated using regression techniques. Because results indicated that slopes may be unique to each water body, pre-impoundment mercury data is needed from each site to make accurate return time predictions.

Analysis of time series data indicated that slopes decreased for 4-5 years following impoundment and then increased back toward pre-impoundment levels. This suggested that bioaccumulation of Hg released from the flooded area to zooplankton could have resulted in more rapid accumulation in younger smaller fish after impoundment than before impoundment. Because these smaller fish are more efficient

accumulators and more sensitive indicators of mercury release, they are the best group to monitor. Further research is needed to substantiate the trend in slopes observed from the time series data. Sites included in the initial slope analysis were of too great post-impoundment age to demonstrate the trend in slope seen from fish in Cat Arm.

Weighted regression analysis indicated that mercury declined with reservoir age for all sites and each species collected across NF. Estimates of return times based on these models are easy to make but require pre-impoundment data. The time series data indicated that pre-impoundment slopes and control pond slopes may be naturally high. Each reservoir may have its own unique starting point making mercury return times unique to each reservoir.

3.7. Acknowledgements:

Thanks are extended to the Green Plan Toxic Contaminants Program that provided the funding for this project. Thanks to C.P. Pennell and K. Ricketts for assistance in collecting water samples; thanks to L. Cole, R. Lawrence, and K. Clarke for assistance collecting fish; thanks to B. Hatfield for running CHN's and Dr. R. Rivkin for use of his fluorometer.

Chapter 4: Summary

Mercury in Recreational Fish from the Province of Newfoundland.

4.1. Summary:

Mercury poisoning in the Lake St. Clair area of Ontario in 1969 lead to investigations into mercury in reservoir fish. Subsequently, elevated mercury levels were found in fish from hydroelectric reservoirs (Bodaly *et al.*, 1984; Hecky *et al.*, 1987; Morrison and Thérien, 1991; Verdon *et al.*, 1994; Montgomery *et al.*, 1995).

Because of the toxicity of mercury and the potential threat to recreational fisheries, empirical models predicting return times have been developed incorporating physical characteristics of reservoirs. Estimates are variable, ranging from as little as 5 years (Abernathy and Cumbie, 1977) to 20-30 years (Verdon *et al.*, 1991). Scruton *et al.* (1994) predicted return times of 7-12 years for salmonids collected from recently (post-1980) created reservoirs in Newfoundland.

Models predicting return times have been primarily based on reservoir age. Factors such as the flooded riparian zone (Zillioux *et al.*, 1993; St. Louis *et al.*, 1994; Krabbenhoft *et al.*, 1995), dissolved organic carbon (Winfrey and Rudd, 1990; Gilmour and Henry, 1991; Miskimmin *et al.*, 1992; Watras *et al.*, 1995), productivity (Wright and Hamilton, 1982; Korthals and Winfrey, 1987), and acidity (Miller and Akagi, 1979; Ramlal *et al.*, 1985; Wiener *et al.*, 1990; Gilmour and Henry, 1991) have all been postulated to affect return times by influencing the availability of methyl mercury to aquatic biota. It was the goal of this study to improve upon predictions of return times for Newfoundland made by Scruton *et al.* (1994) by expanding the current database of mercury in fish and by incorporating several of these factors that are postulated to affect mercury return times.

An investigation into sediment mercury levels in 34 non-impounded headwater lakes across NF indicated that acidity had no effect on the amount of mercury accumulated at the lake bottom. Watershed area to lake area ratio (WA:LA) was found to be significant in explaining the variation observed in sediment mercury levels. This suggested that large watersheds can deposit significant amounts of mercury sorbed to organic material to small lakes that then act as sinks for this metal. This relationship was further supported by the positive correlation between lake colour and WA:LA.

The expanded mercury database incorporated data from 16 reservoirs and three control ponds. The slope of the relationship between $\log_{10}$ (Hg (ppm)) and $\log_{10}$ (Fork length (cm)) was found to change significantly over time and so was used to evaluate the temporal evolution of mercury in hydroelectric reservoirs. This analysis indicated that for ouananiche and brook trout the slope was a function of the age of the reservoir and water conductivity. The relationship with age supported the notion that elevated mercury in fish following impoundment is transitory: the relationship with conductivity suggested that methyl mercury uptake from the water column due to a lack of competing ions may be important in NF reservoirs. Slope values observed for arctic charr were a function of the % Area Flooded and this pointed to the inundated area as a source of mercury to reservoir fish.

Slopes varied among control ponds. This precluded determination of return times for either species because of difficulty determining what represented a background mercury level. To investigate slope variability, a time series of mercury in fish data from a select number of reservoirs operated by Newfoundland and Labrador Hydro was

analysed. This analysis suggested that impoundment lowered the slope for the first 3-4 years following impoundment. Slopes then started to return to pre-impoundment levels. In the absence of pre-impoundment data it is not clear whether this relationship applies to other water bodies.

Time series data from control ponds indicated that slopes from unimpounded sites can be similar to or greater than reservoir slopes suggesting that each reservoir may have its own unique pre-impoundment slope and return time. Time series data also suggested that fish at the lower trophic levels received the greatest amount of mercury following inundation. Therefore, this appears to be the best group to monitor to determine the impact of impoundment on resident fish populations.

To make predictions of return time for the three species collected from reservoirs across Newfoundland, weighted regression was used incorporating reservoir age and mercury data from a preselected size range. For ouananiche, brook tout, and arctic charr, mercury increase and decline were found to be a significant function of reservoir age. In each case, the increase of post-impoundment mercury during the first few yeas following inundation was much higher than the subsequent rate of decline.

This study shows that mercury contamination is a widespread problem that may apply to all water bodies on the island and not just hydroelectric developments. Because fish part of natural systems are carrying high mercury body burdens, mercury contamination may be part of a larger global and anthropogenic problem.

Chapter 5: References

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Chapter 6: Appendices

Appendix 1: Linear Regression Plots

A.

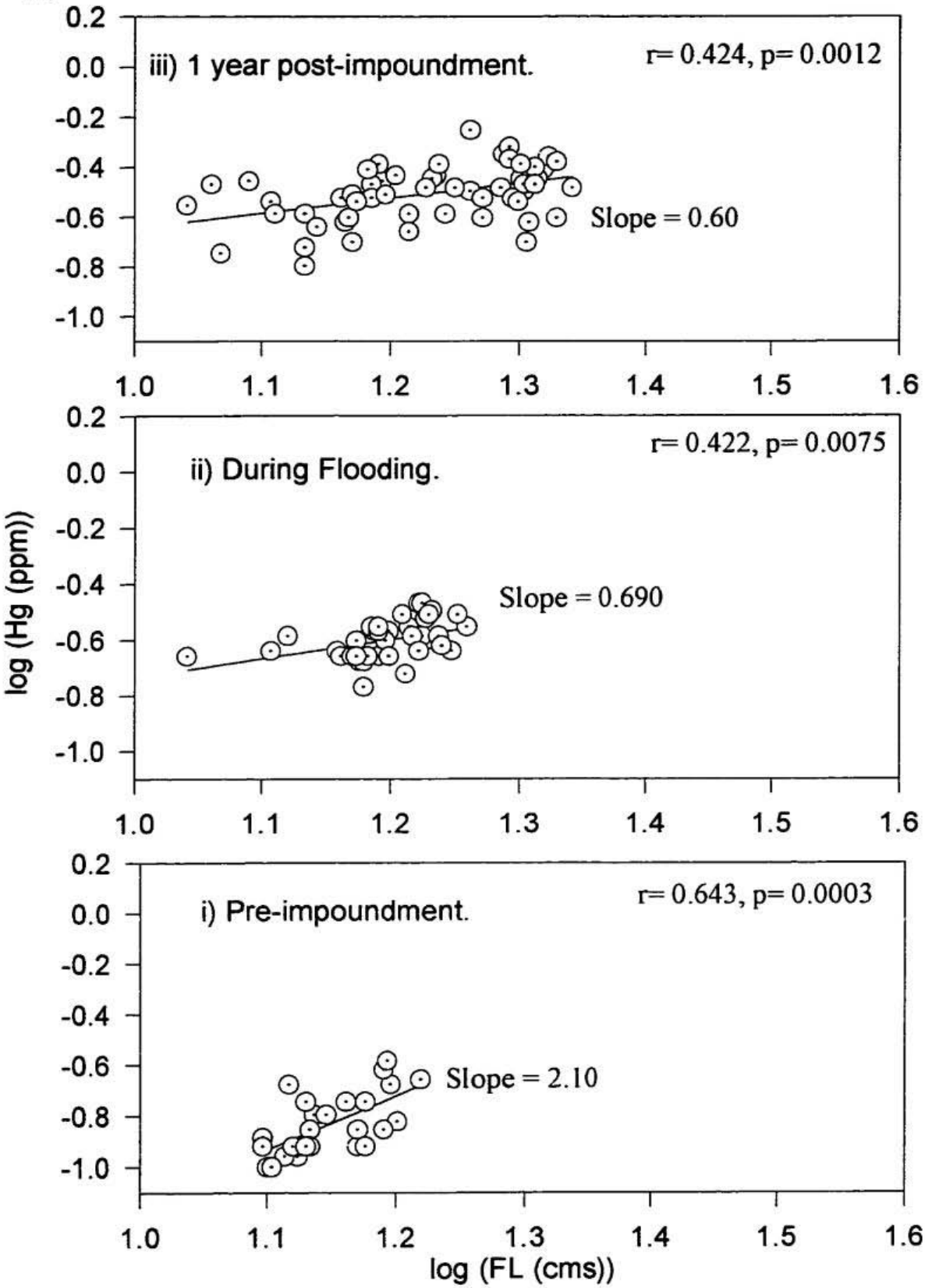


Figure A- 1a.: Plots of log (Hg (ppm)) vs log (FL (cm) for arctic charr from Cat Arm Reservoirs for i) pre-impoundment, ii) during impoundment, and iii) 1 year post-impoundment. Solid line represents the least squares regression.

B.

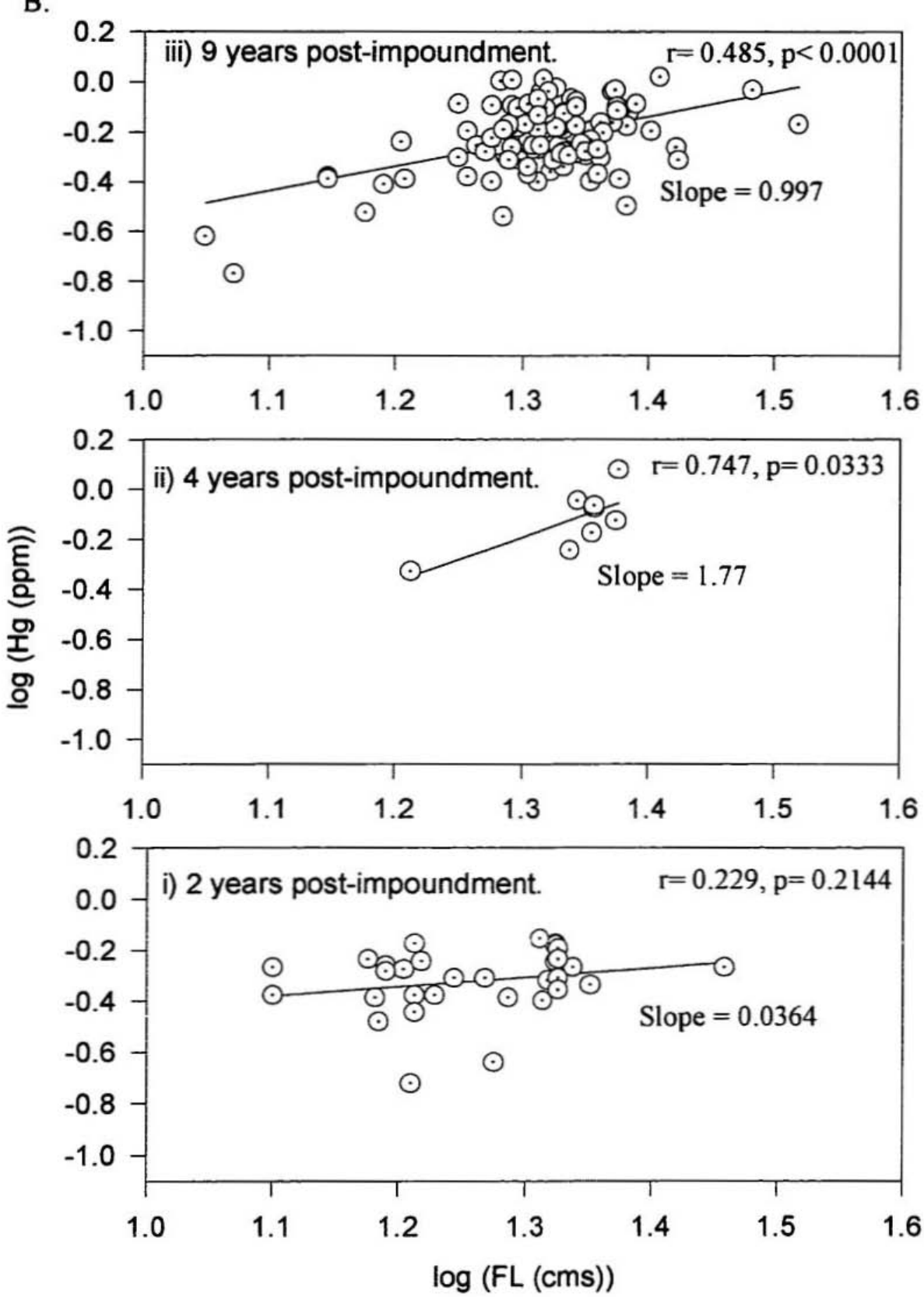


Figure A- 1b.: Plots of log (Hg (ppm)) vs log (FL (cm)) for arctic charr from Cat Am reservoir for i) post-impoundment 1986, ii) post-impoundment 1988, and iii) post-impoundment 1993. Solid line represents the least square regression.

A.

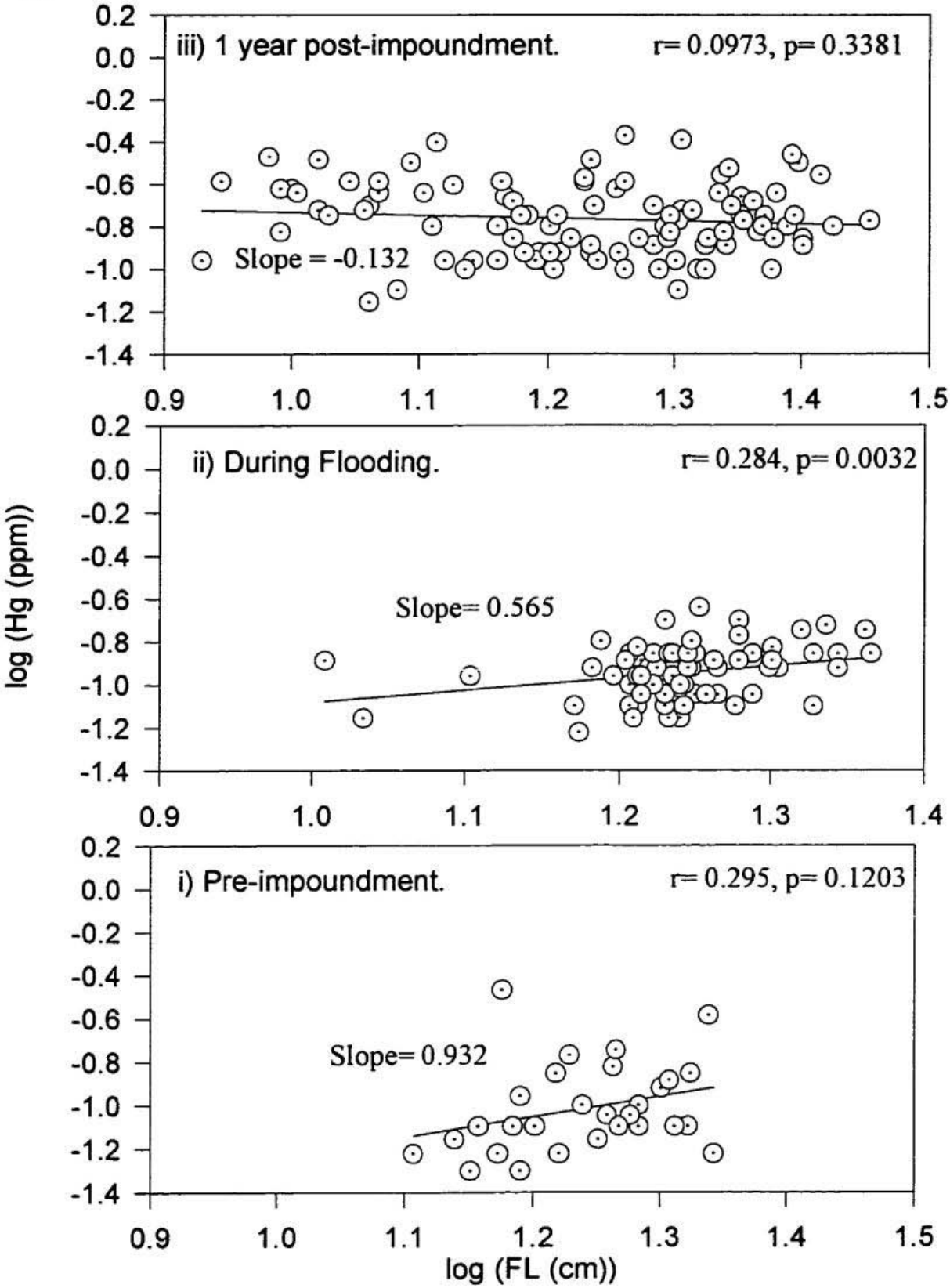


Figure A- 2a.: Plots of log Hg (ppm) vs log FL (cm) for brook trout collected from Cat Arm reservoir for i) pre-impoundment, ii) during impoundment, and iii) 1 year post-impoundment. Solid lines represent the least squares regression.

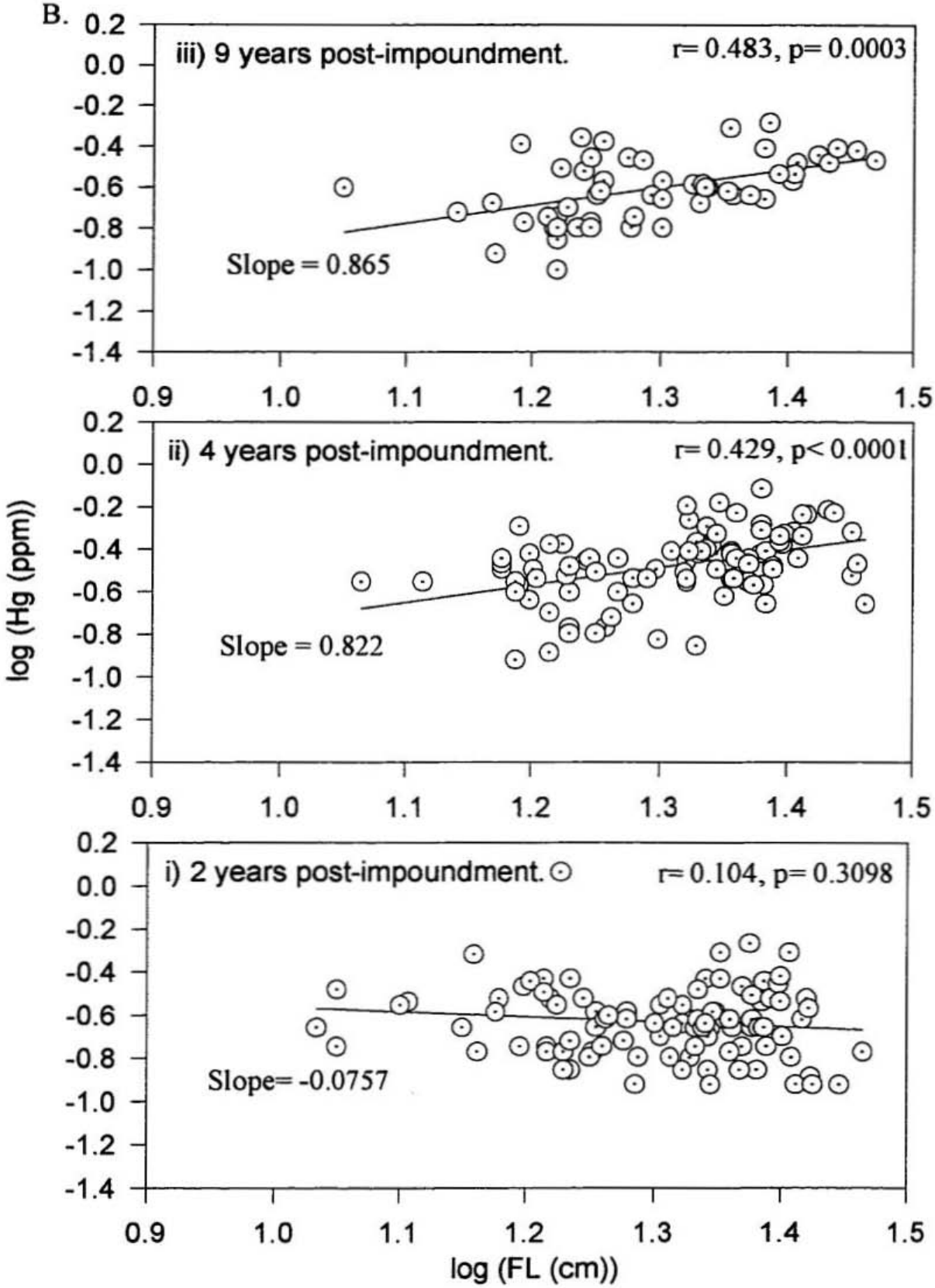


Figure A- 2b.: Plots of log (Hg (ppm)) vs log (FL (cm)) for brook trout collected from Cat Arm Reservoir for i) 2, ii) 4, and iii) 9 years post-impoundment. Solid lines represent the least squares regression.

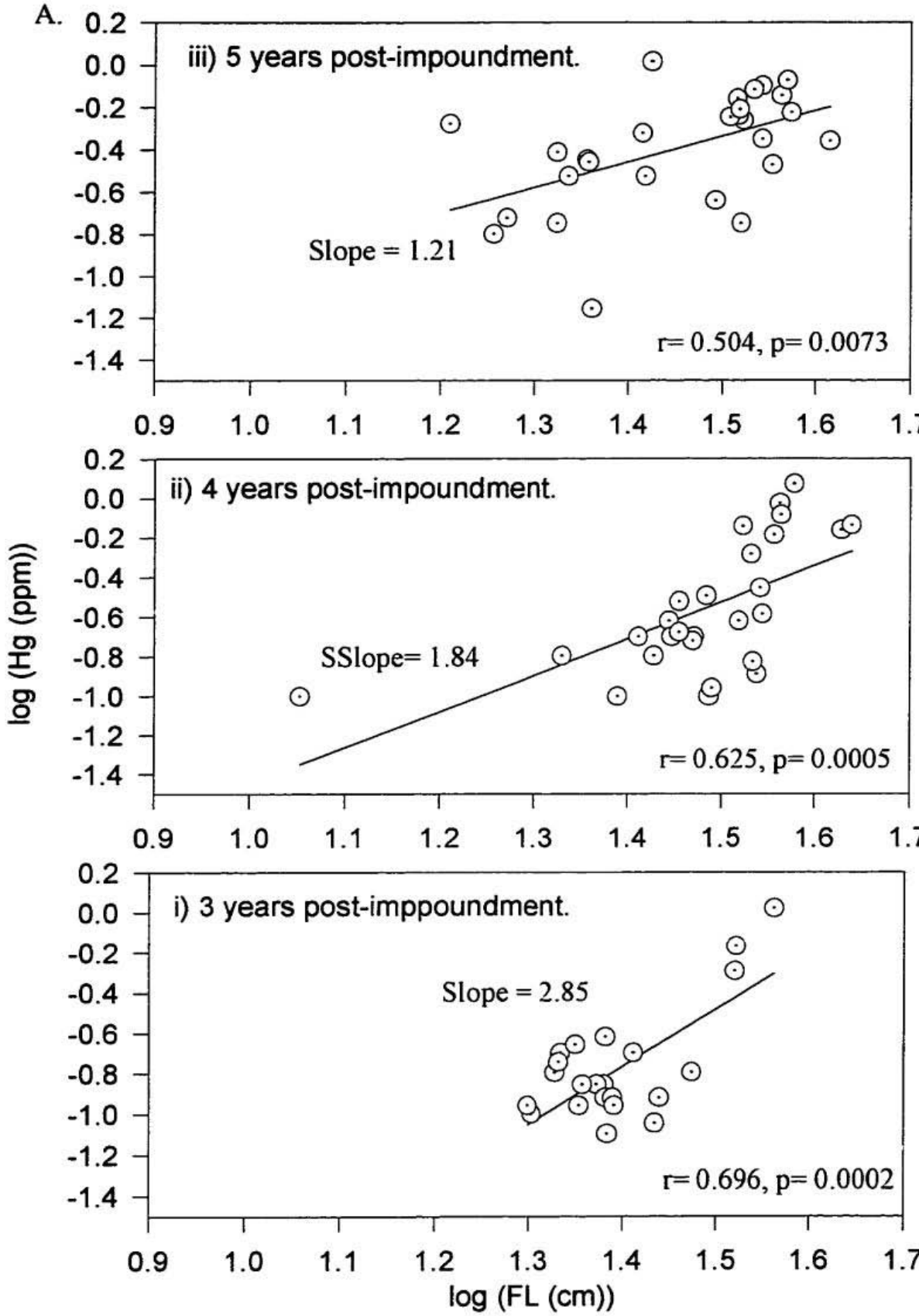


Figure A- 3a.: Plots of log (Hg (ppm)) vs log (Fl (cm)) for brook trout collected from Hind's Lake Reservoir for i) 3, ii) 4, and iii) 5 years post-impoundment. Solid lines represent the least squares regression.

B.

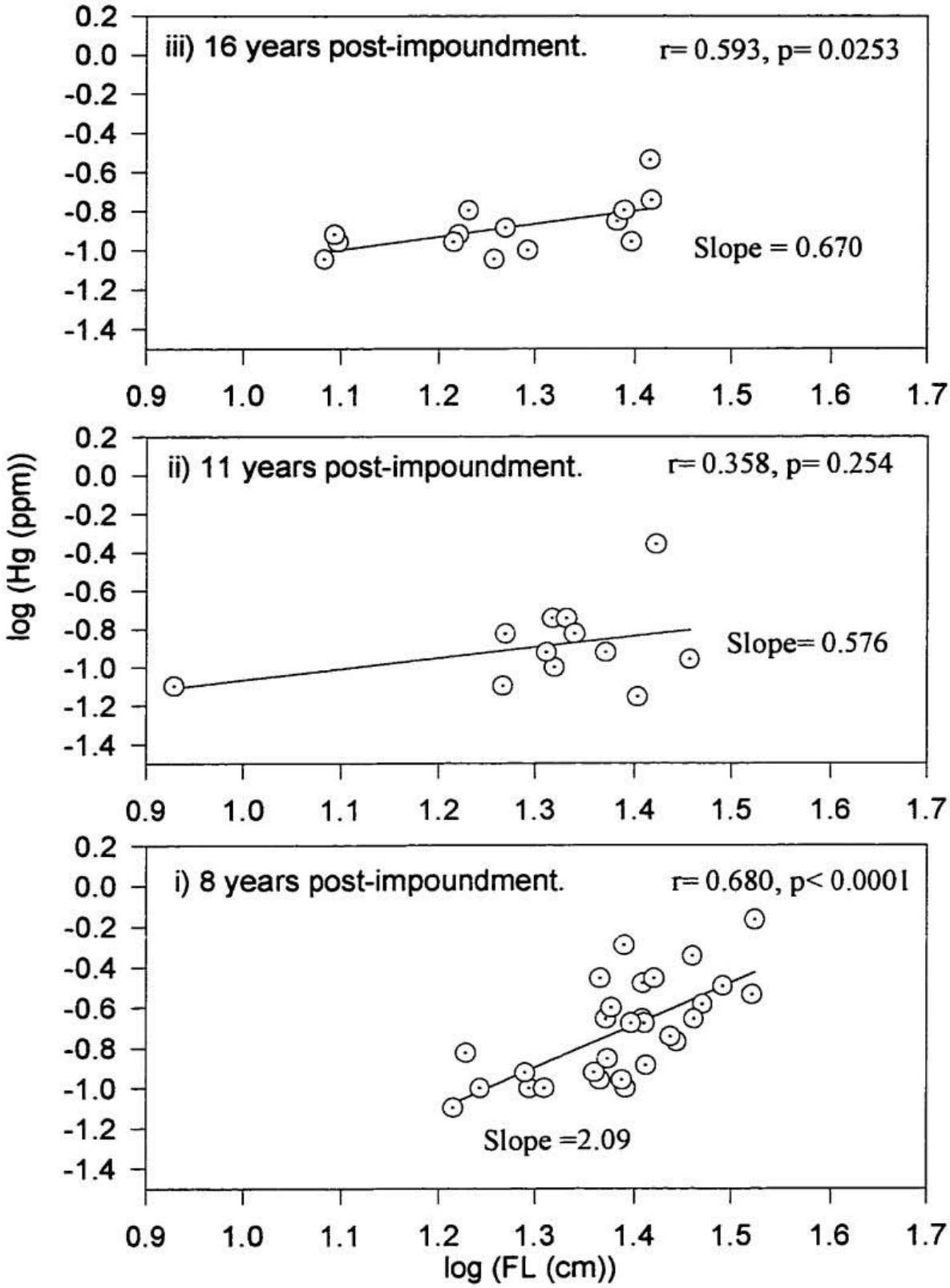


Figure A- 3b.: Plots of log (Hg (ppm)) vs log (FL (cm)) for brook trout collected from Hind's Lake Reservoir for i) 8, ii) 11, and iii) 16 years post-impoundment. Solid lines represent the least squares regression.

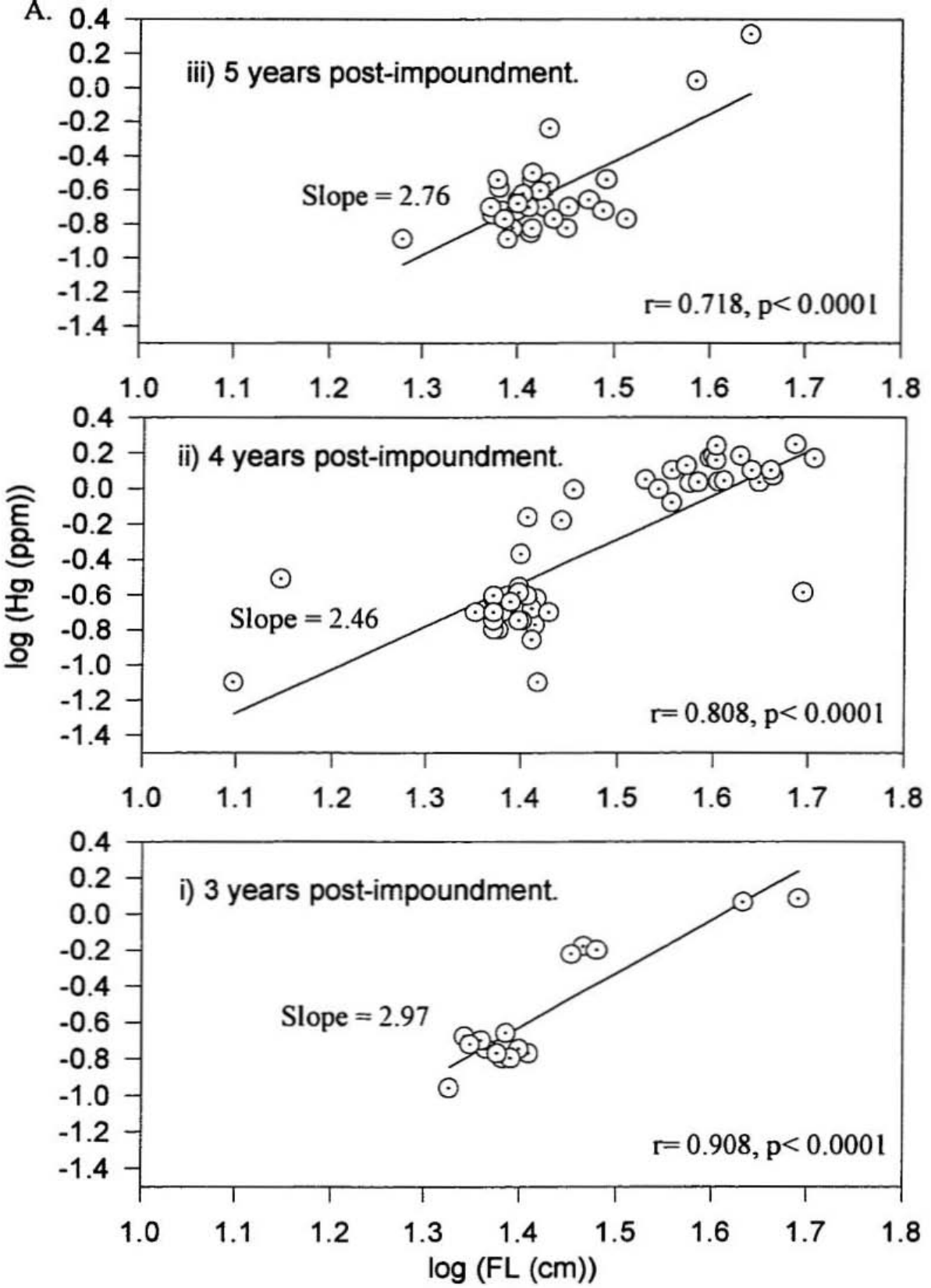


Figure A- 4a.: Plots of log (Hg (ppm)) vs log (FL (cm)) for ouananiche from Hind's Lake Reservoir for i) 3, ii) 4, and iii) 5 years post-impoundment. Solid \ lines represent the least squares regression.

B.

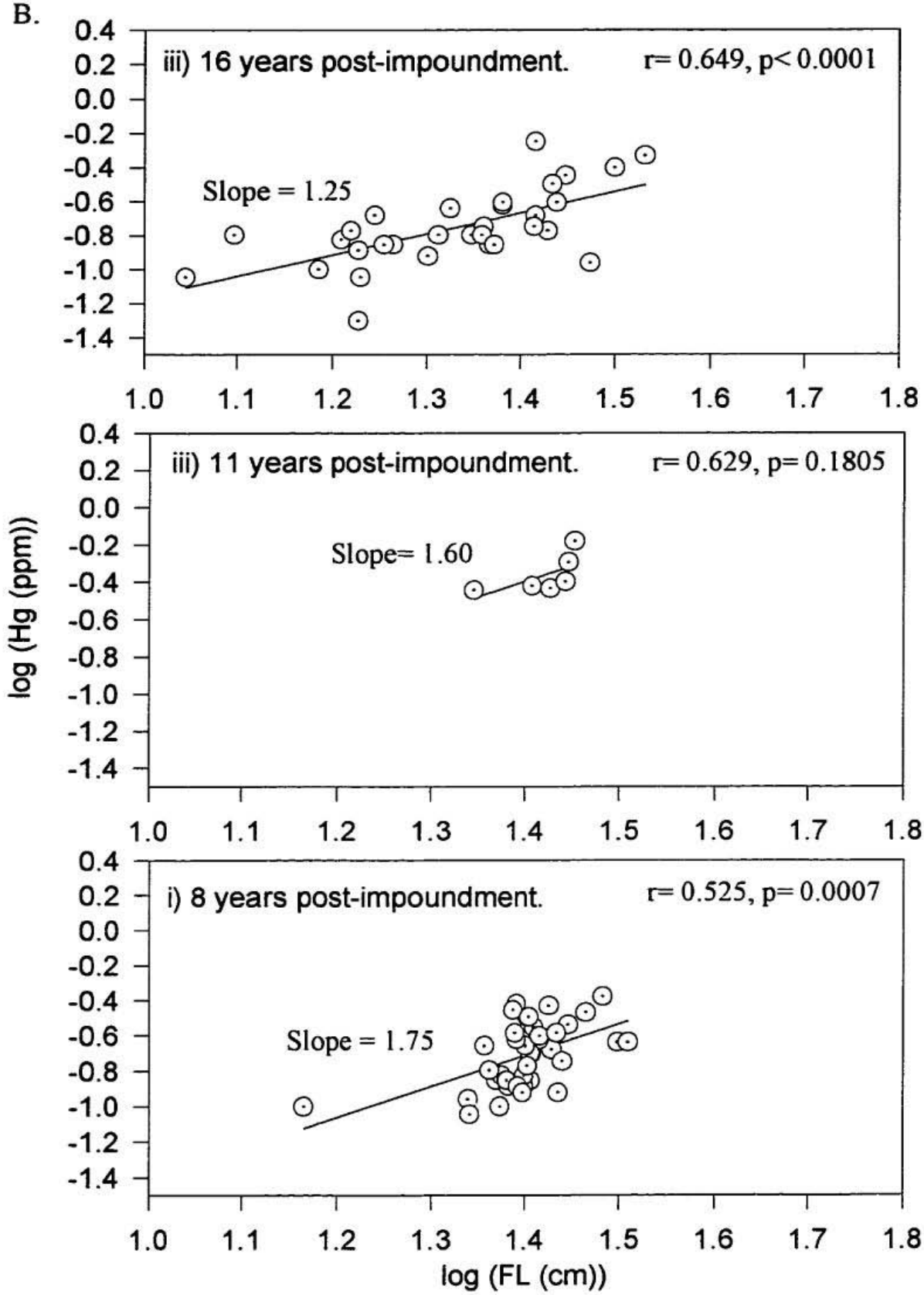


Figure A- 4b.: Plots of log (Hg (ppm)) vs log (FL (cm)) for ouananiche collected from Hind's Lake reservoir for i) 8, ii) 11, and iii) 16 years post-impoundment. Solid lines represent the least squares regressions.

A.

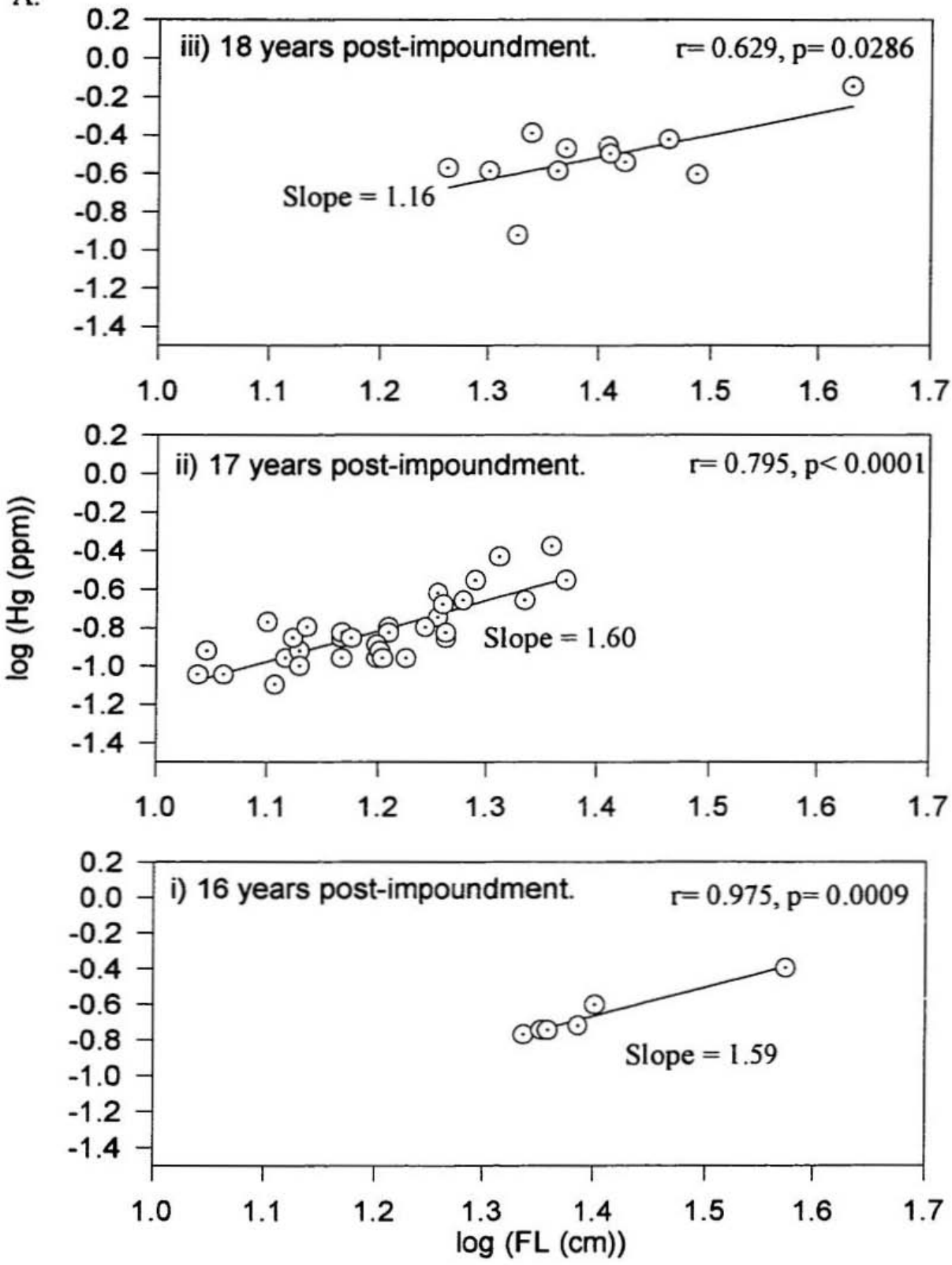


Figure A- 5a.: Plots of log (Hg (ppm)) vs log (FL (cm)) for brook trout collected from Long Pond Reservoir for i) 16, ii) 17, and iii) 18 years post-impoundment. Solid lines represent the least squares regressions.

B.

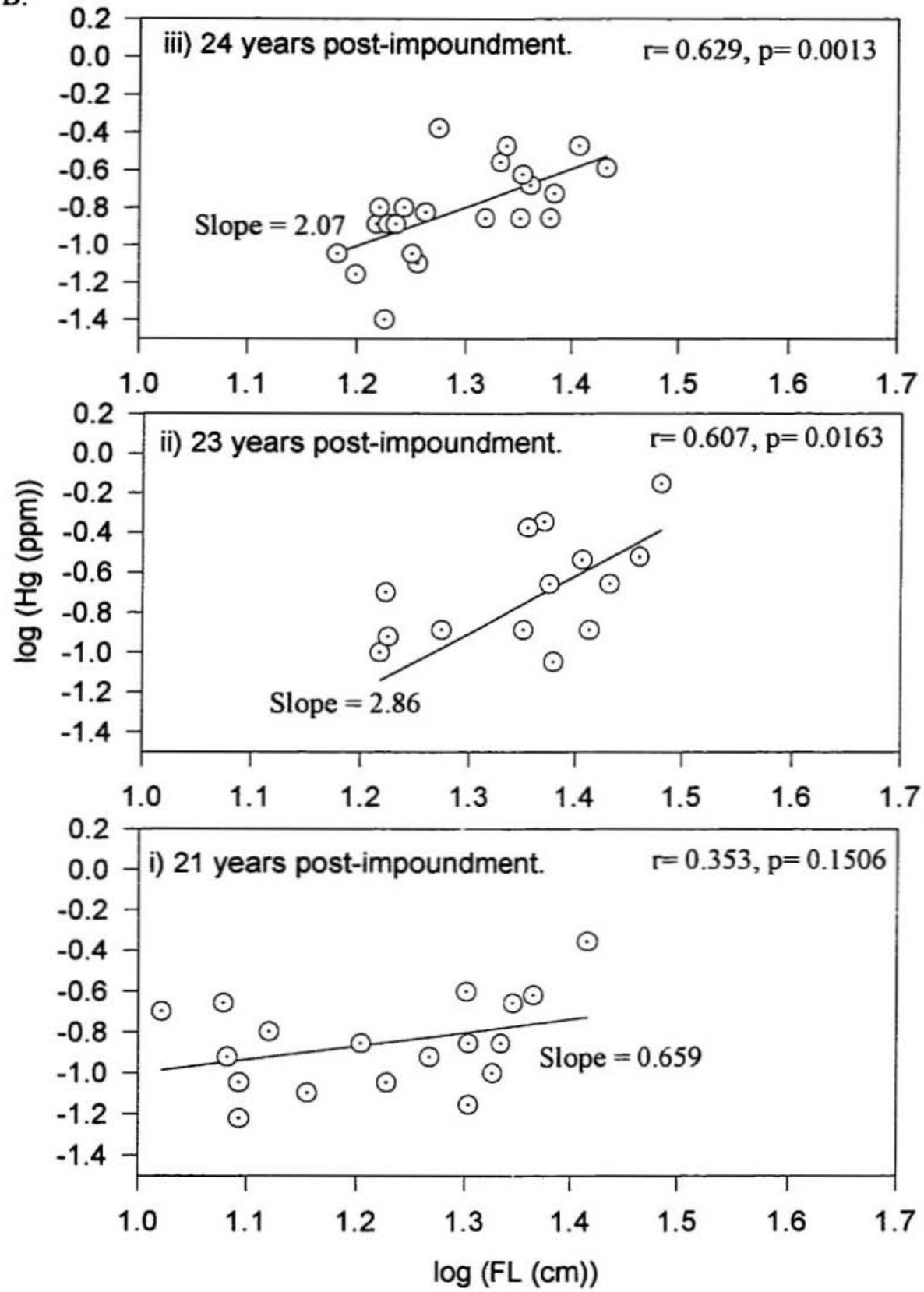


Figure A- 5b. : Plots of log (Hg (ppm)) vs log (FL (cm)) for brook trout collected from Long Pond Reservoir for i) 21, ii) 23, and iii) 24 years post-impoundment. Solid lines represent the least squares regressions.

C.

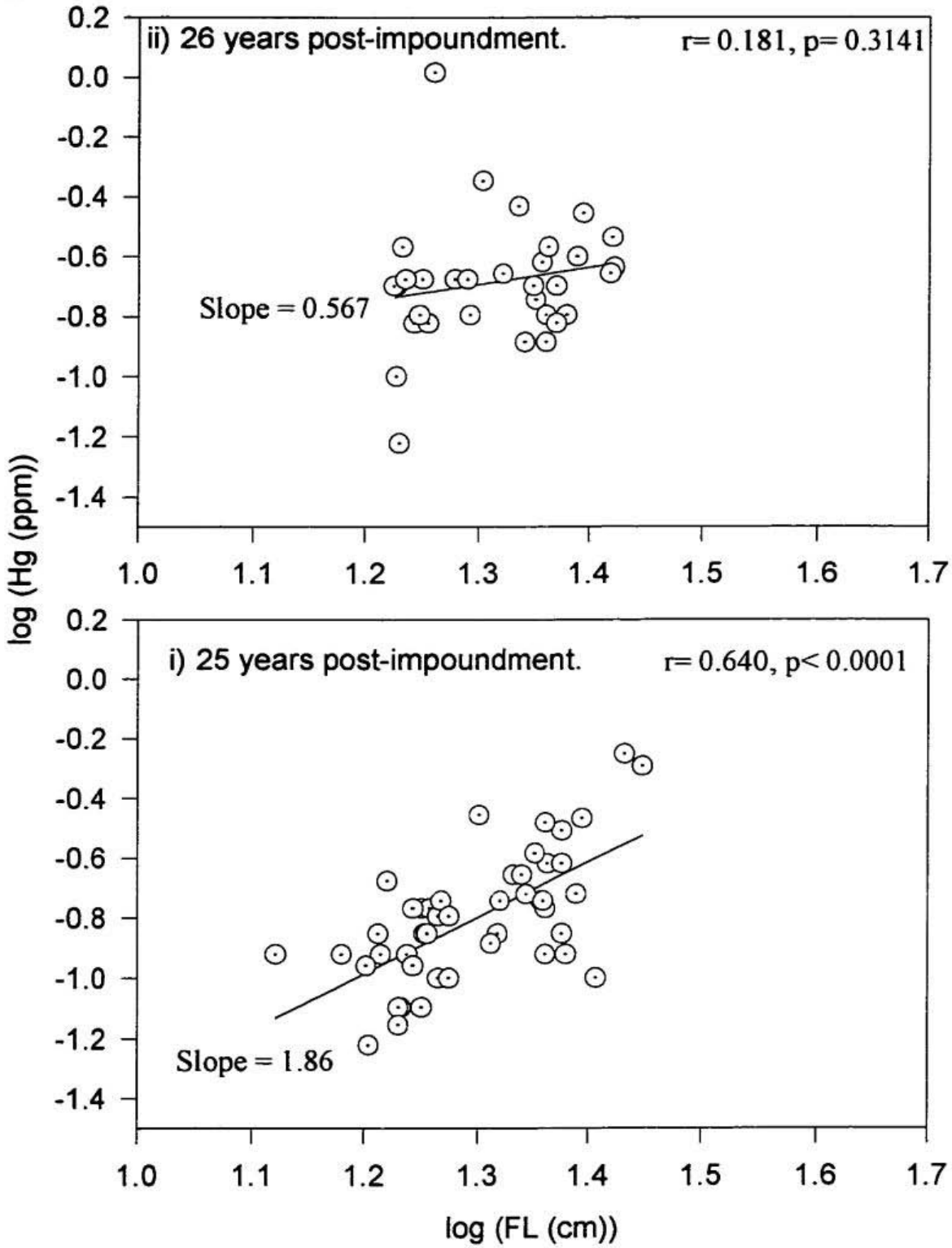


Figure A- 5c.: Plots of log (Hg (ppm)) vs log (FL (cm)) for brook trout collected from Long Pond Reservoir for i) 25 and ii) 26 years post-impoundment. Solid lines represent the least squares regressions.

A.

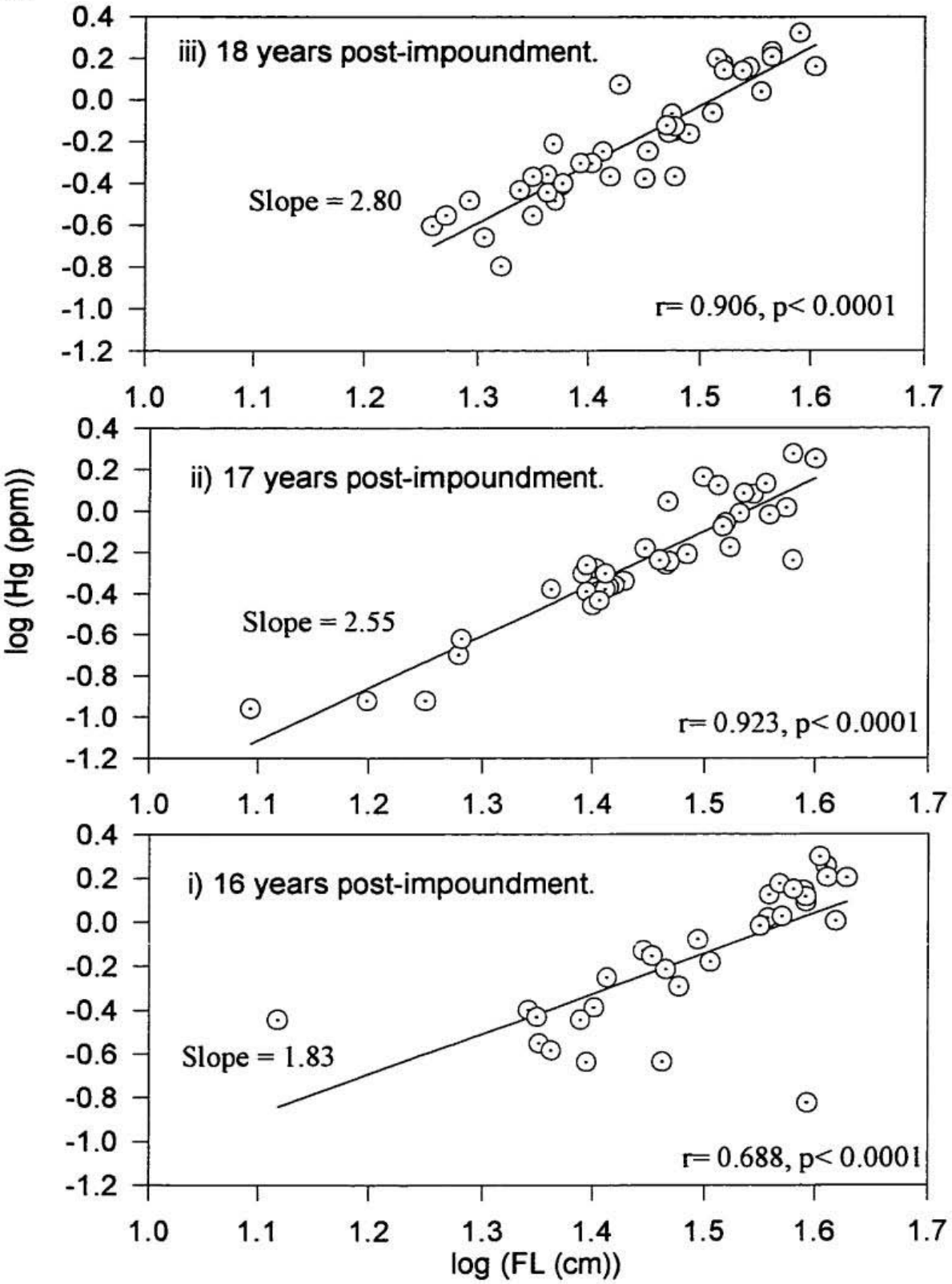


Figure A- 6a.: Plots of log (Hg (ppm)) vs log FL (cm)) for ouananiche collected from Long Pond Reservoir for i) 16, ii) 17, and iii) 18 years post-impoundment. Solid lines represent the least squares regressions.

B.

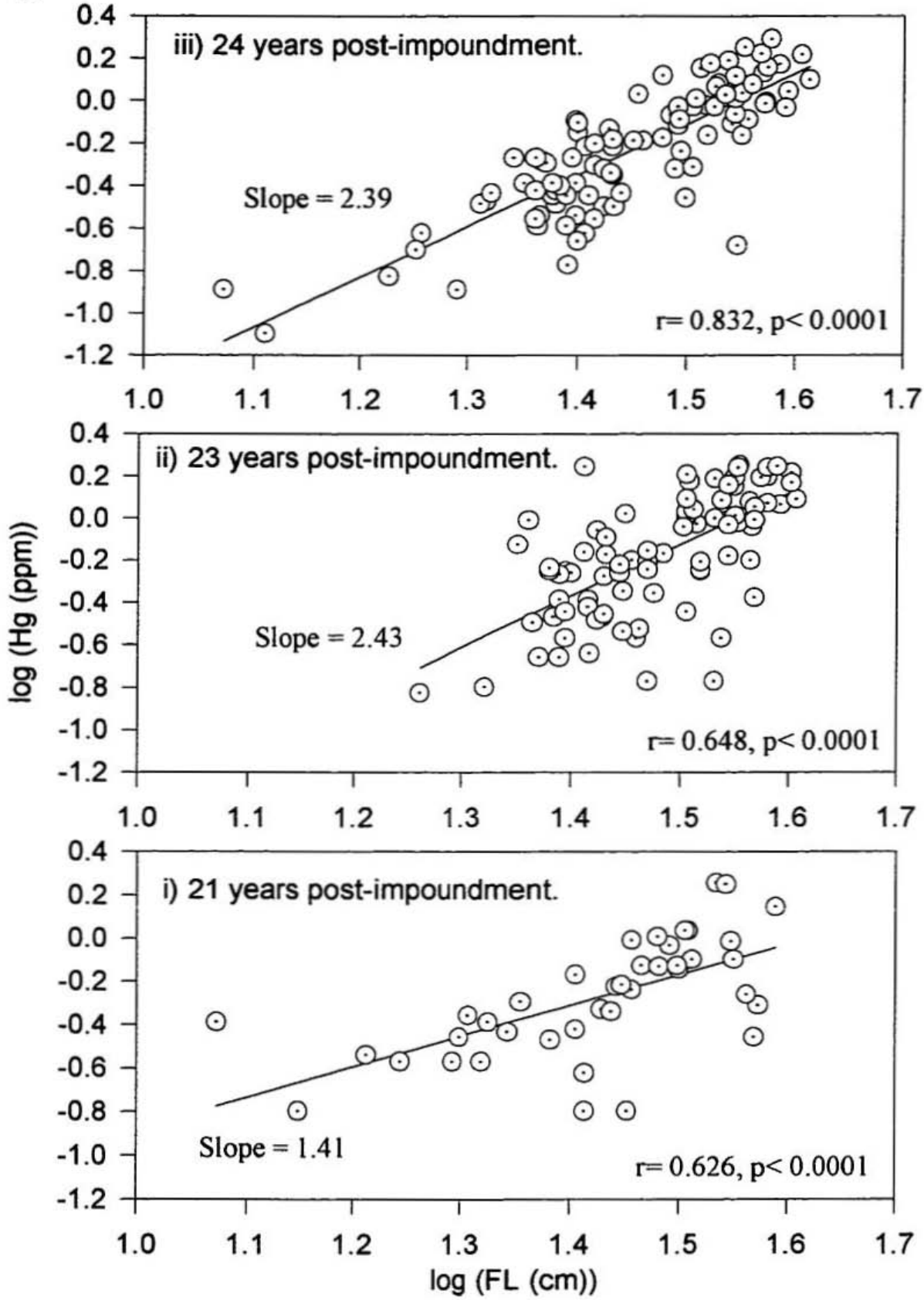


Figure A- 6b.: Plots of log (Hg (ppm)) vs log (FL (cm)) for ouananiche collected from Long Pond Reservoir for i) 21 , ii) 23, and iii) 24 years post-impoundment. Solid lines represent the least squares regressions.

C.

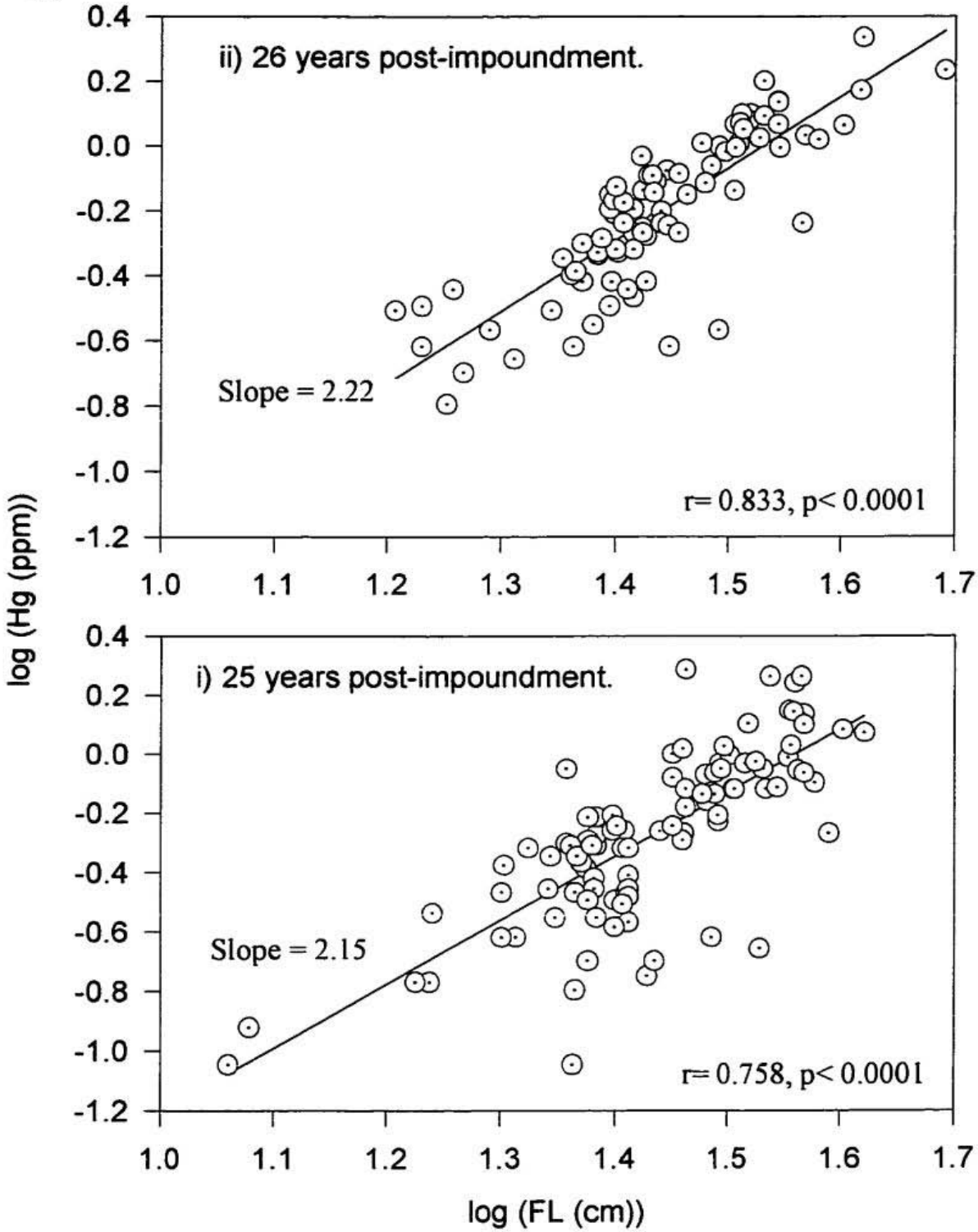


Figure A- 6c: Plots of log (Hg (ppm)) vs log (FL (cm)) for ouanancihe collected from Long Pond Reservoir for i) 25 and ii) 26 years post-impoundment. Solid lines represent the least squares regressions.

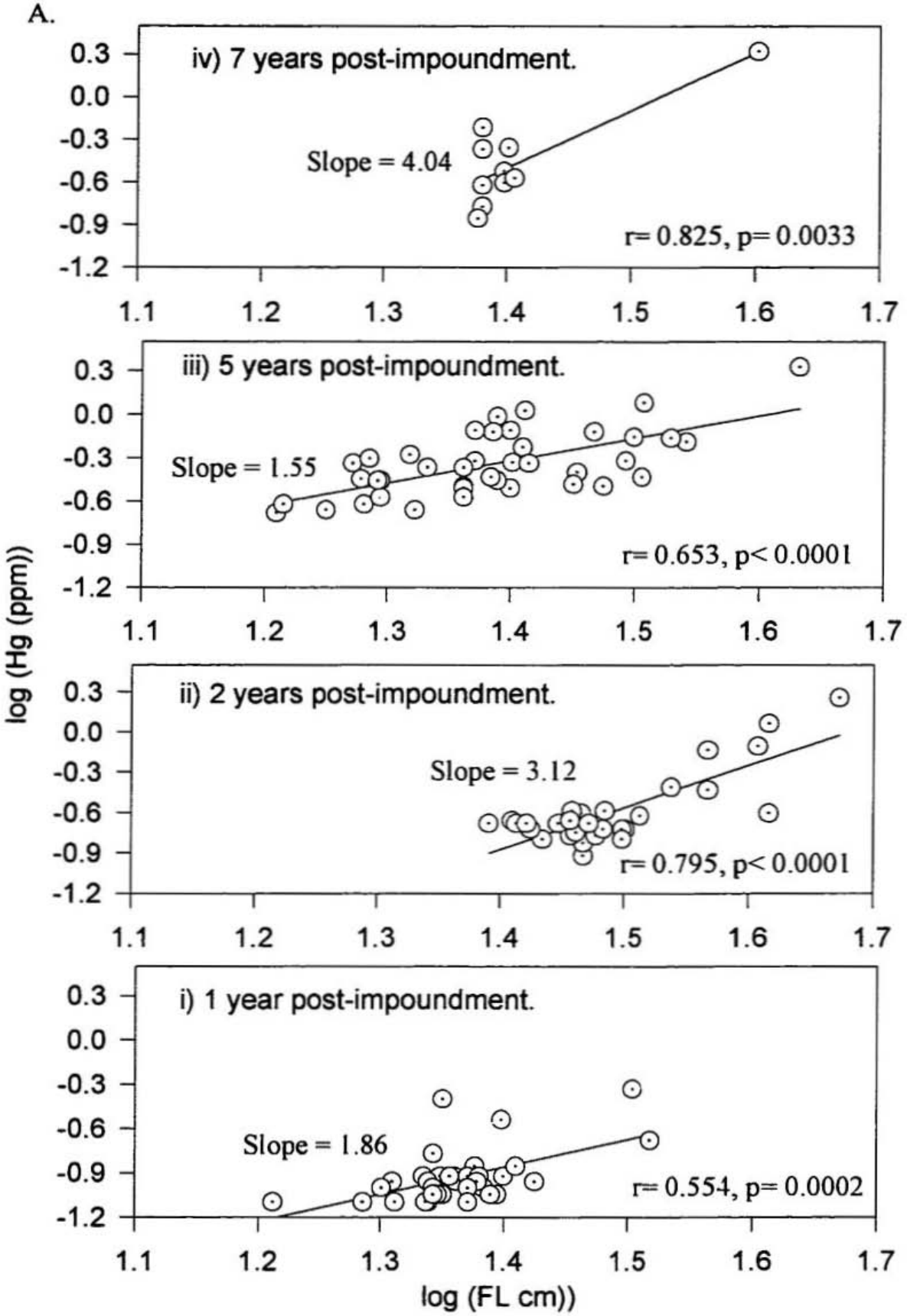


Figure A- 7a.: Plots of log (Hg (ppm)) vs log (FL (cm)) for brook trout from Cold Spring Pond Reservoir for i) 1 year, ii) 2 years, iii) 5 years, and iv) 10 years post-impoundment. Solid lines represent the least squares regressions.

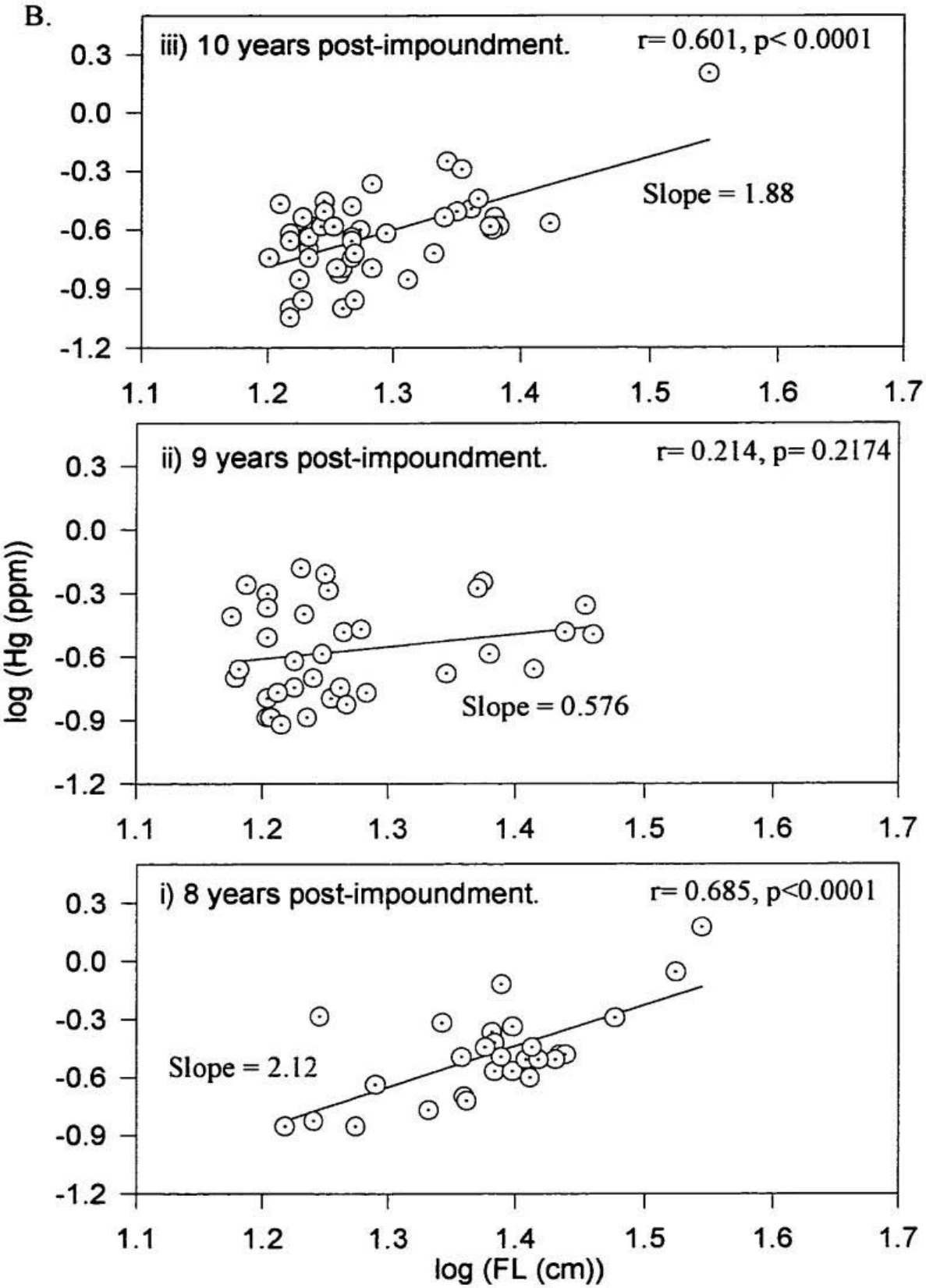


Figure A- 7b.: Plots of log (Hg (ppm)) vs log (FL (cm)) for brook trout from Cold Spring Pond reservoir for i) 8, ii) 9, and iii) 10 years post-impoundment. Solid lines represents the least squares regressions.

A.

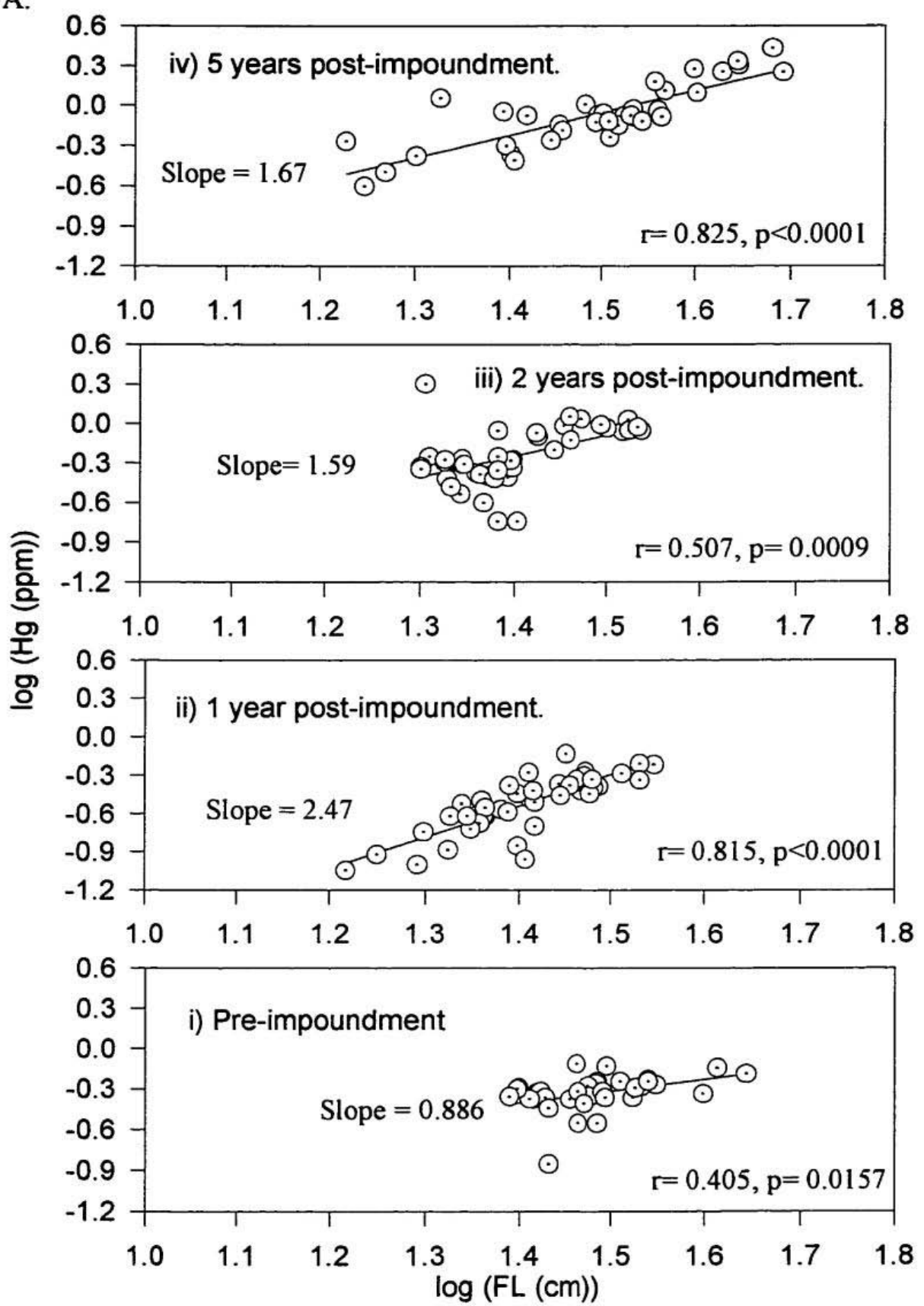


Figure A- 8a.: Plots of log (Hg (ppm)) vs log (FL (cm)) for ouananiche from Cold Spring Pond Reservoir for i) pre-impoundment, ii) 1, iii) 2, and iv) 5 years post-impoundment. Solid lines represent the least squares regressions.

B.

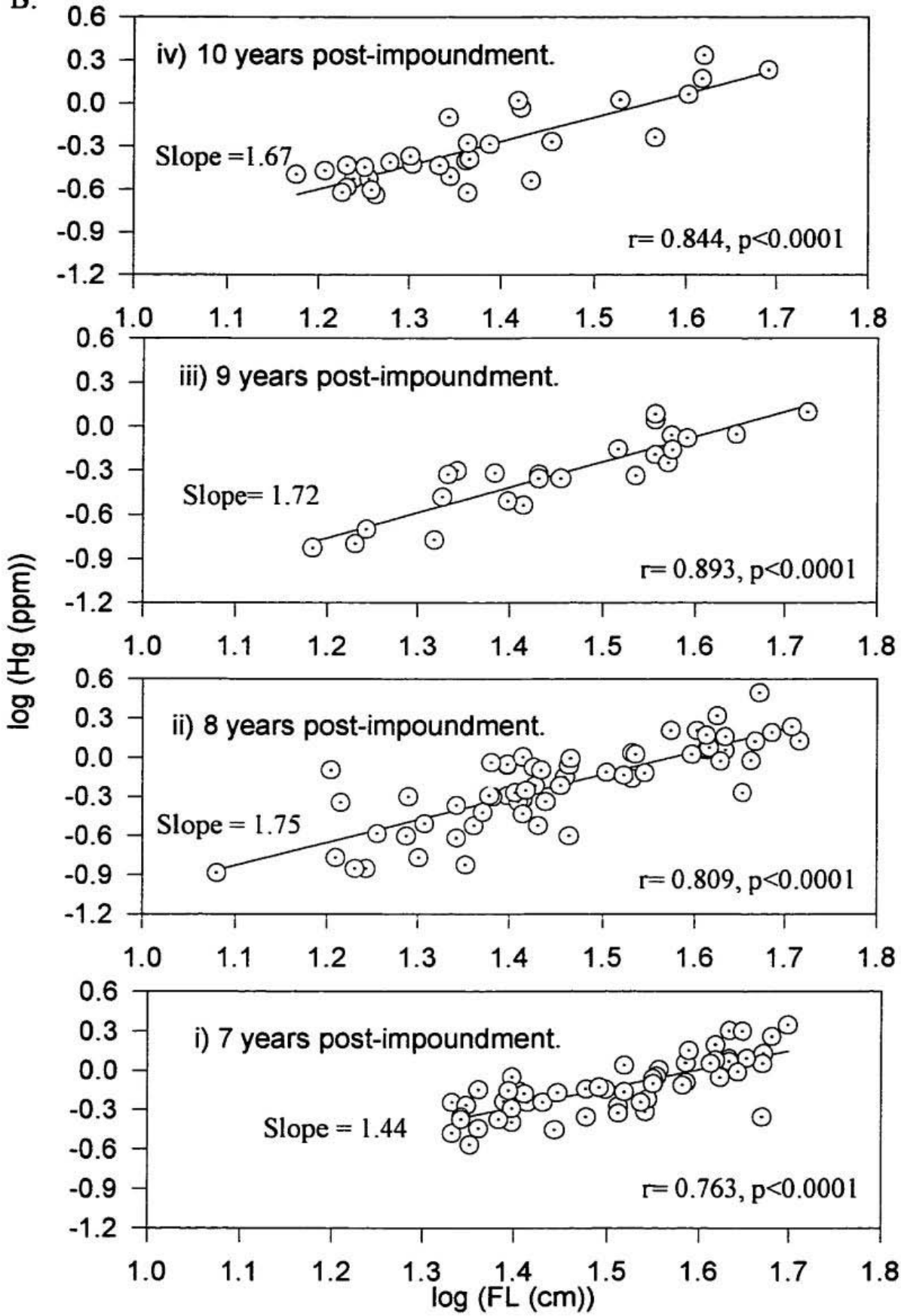


Figure A- 8b.: Plots of log (Hg (ppm)) vs log (FL (cm)) for ouananiche from Cold Spring Pond Reservoir for i) 7, ii) 8, iii) 9, and iv) 10 years post-impoundment. Solid lines represent the least squares regressions.

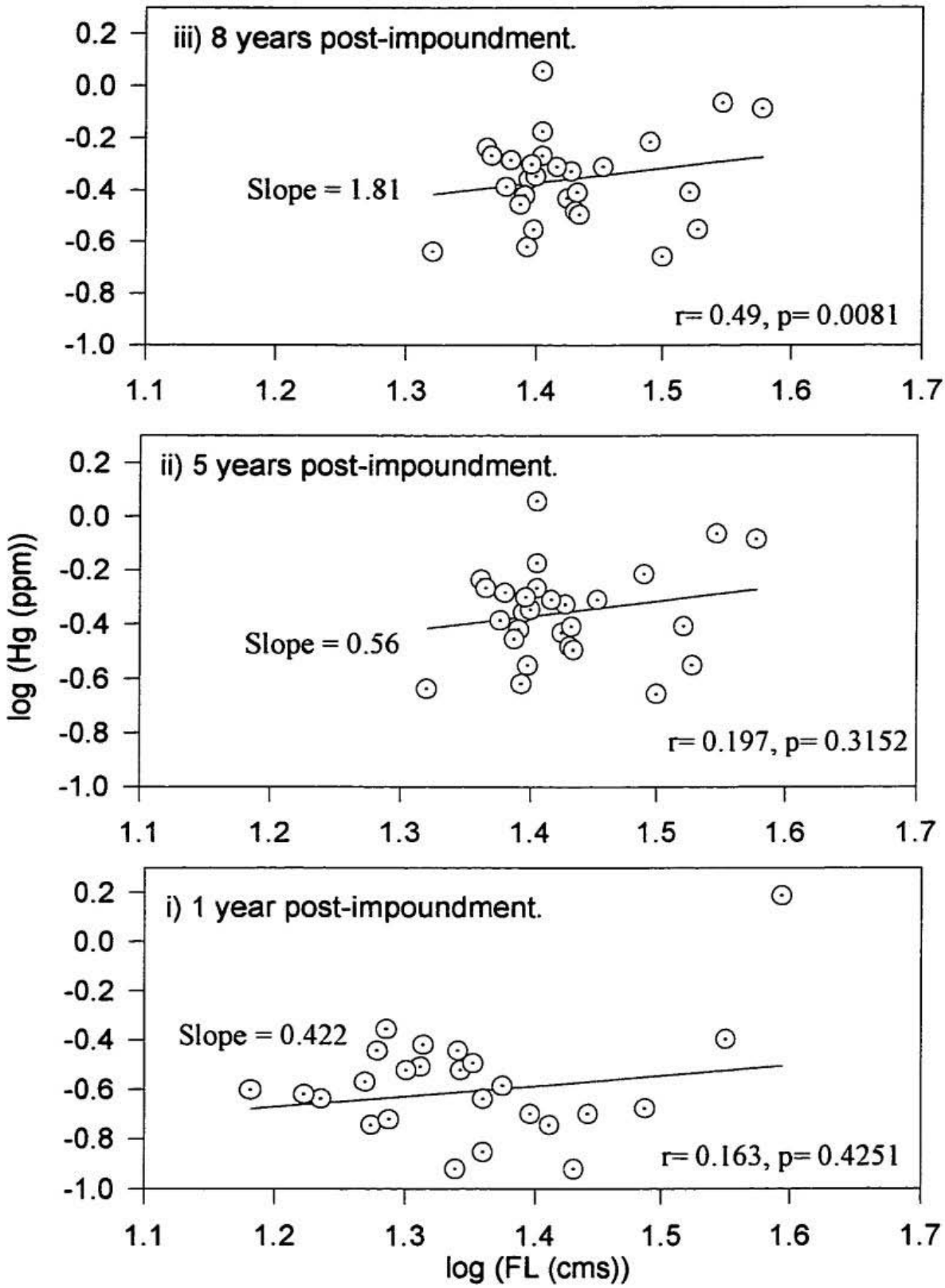


Figure A- 9.: Plots of log (Hg (ppm)) vs log (FL (cm)) for brook trout collected from Great Burnt Lake reservoir for i) 1, ii) 5, and iii) 8 years post-impoundment. Solid lines represent the least squares regressions.

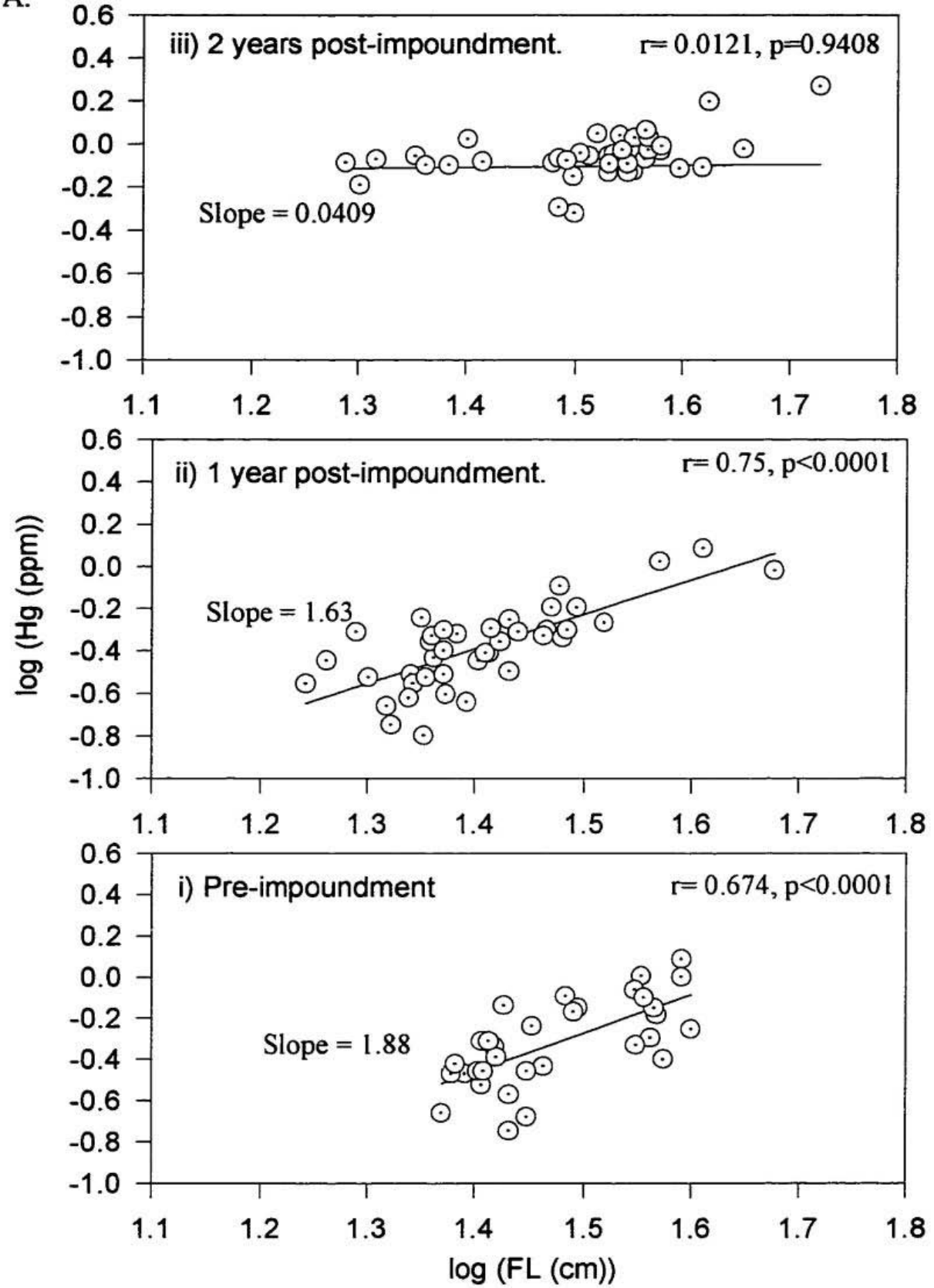


Figure A- 10a.: Plots of log (Hg (ppm)) vs log (FL (cm)) for ouananiche collected from Great Burnt Lake reservoir for i) pre-impoundment, and ii) 1 year and iii) 2 years post-impoundment. Solid lines represent the least squares regressions.

B.

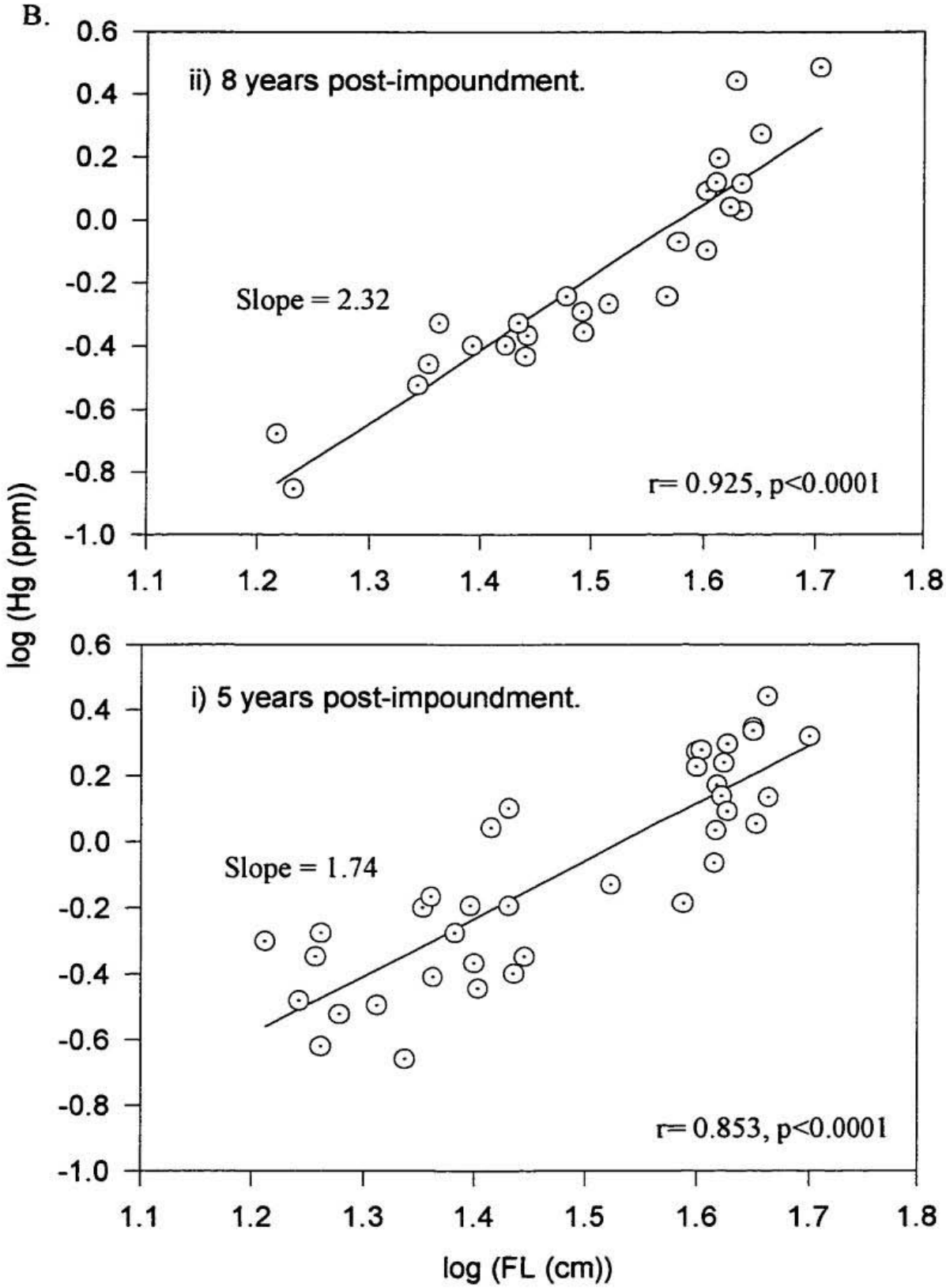


Figure A- 10b.: Plots of log (Hg (ppm)) vs log (FL (cm)) for ouananiche collected from Great Burnt Lake Reservoir for i) 7 and ii) 10 years post-impoundment. Solid lines represent the least squares regressions.

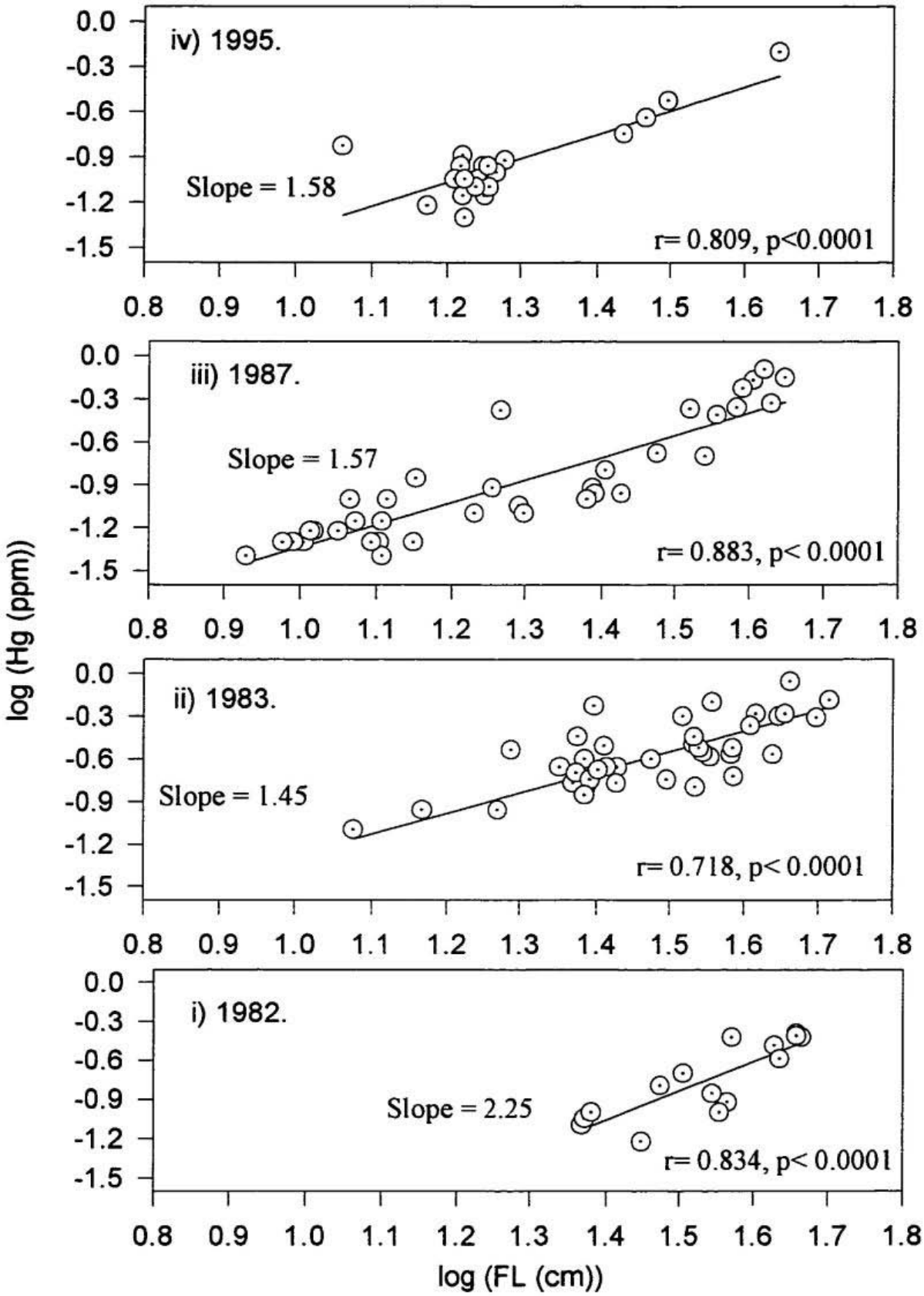


Figure A- 11.: Plots of log (Hg (ppm)) vs log (FL (cm)) for brook trout collected from control site Eclipse Pond for i) 1983, ii) 1984, iii) 1987, and iv) 1995. Solid lines represent least squares regressions.

A.

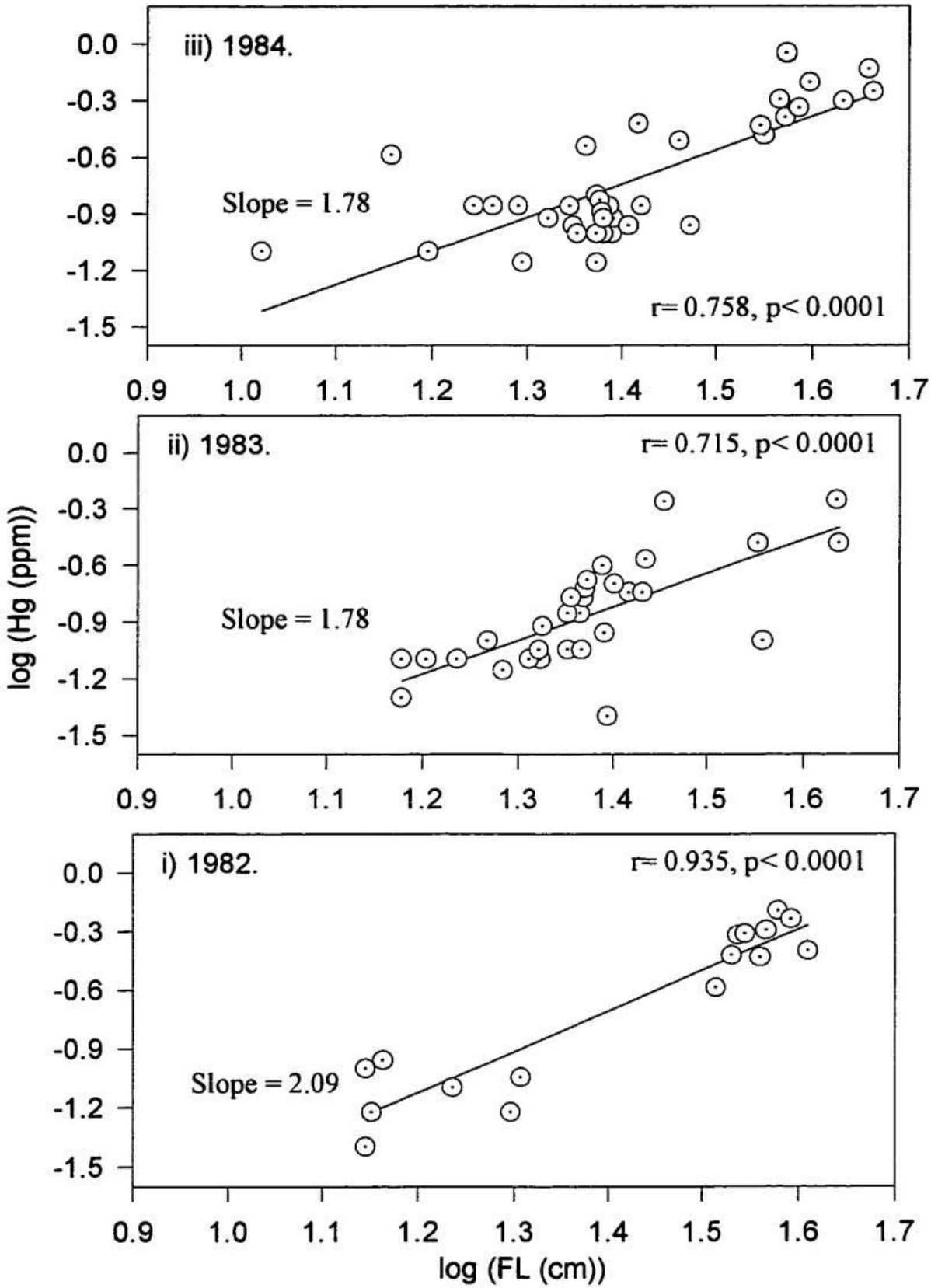


Figure A- 12a.: Plots of log (Hg (ppm)) vs log (FL (cm)) for ouananiche collected from control site Eclipse Pond for i) 1982, ii) 1983, and iii) 1984. Solid lines represent least squares regressions.

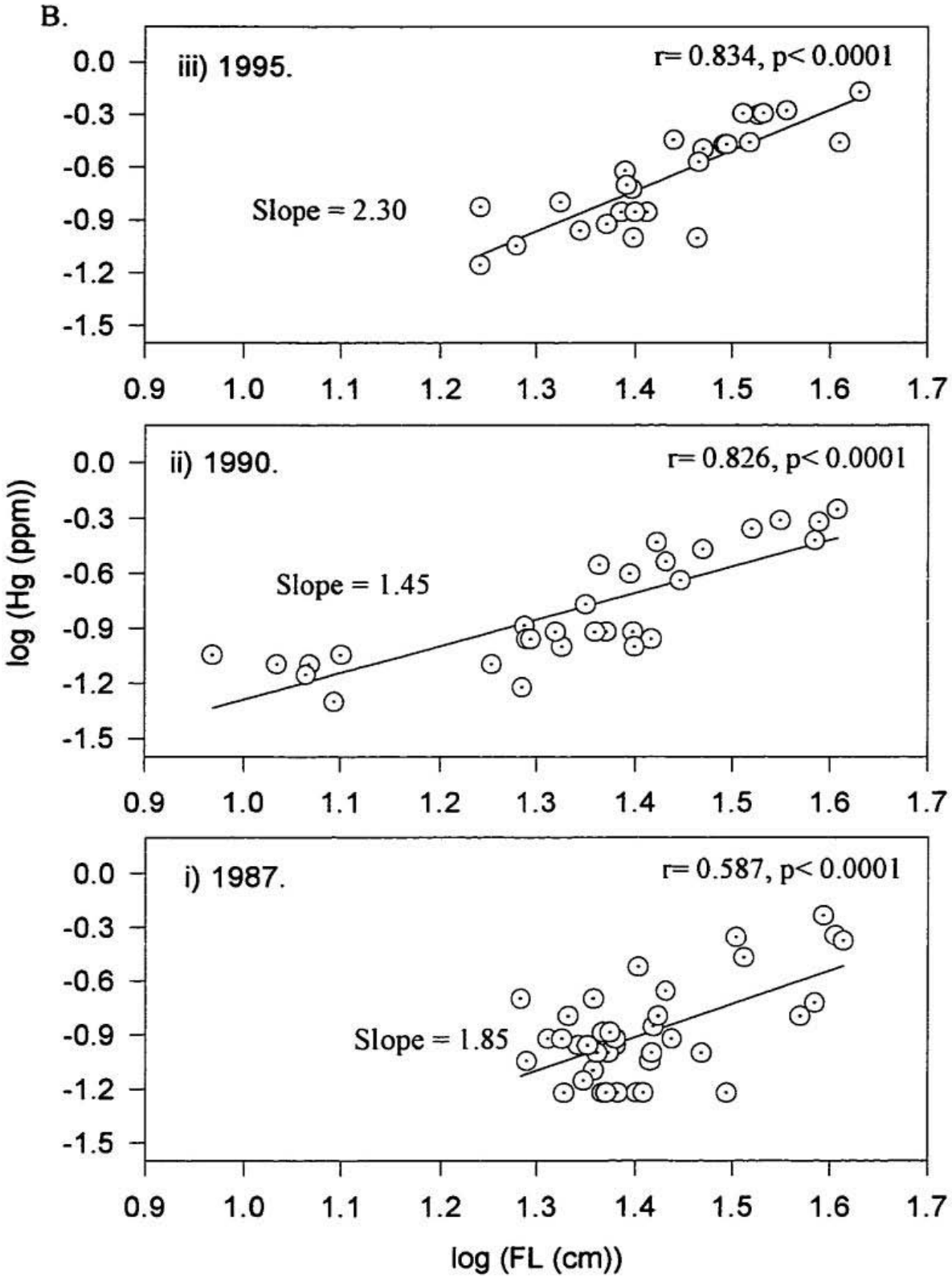


Figure A- 12b.: Plots of log (Hg (ppm)) vs log (FL (cm)) for ouananiche collected from control site Eclipse Pond during years i) 1987, ii) 1990, and iii) 1995. Solid lines represent the least squares regressions.

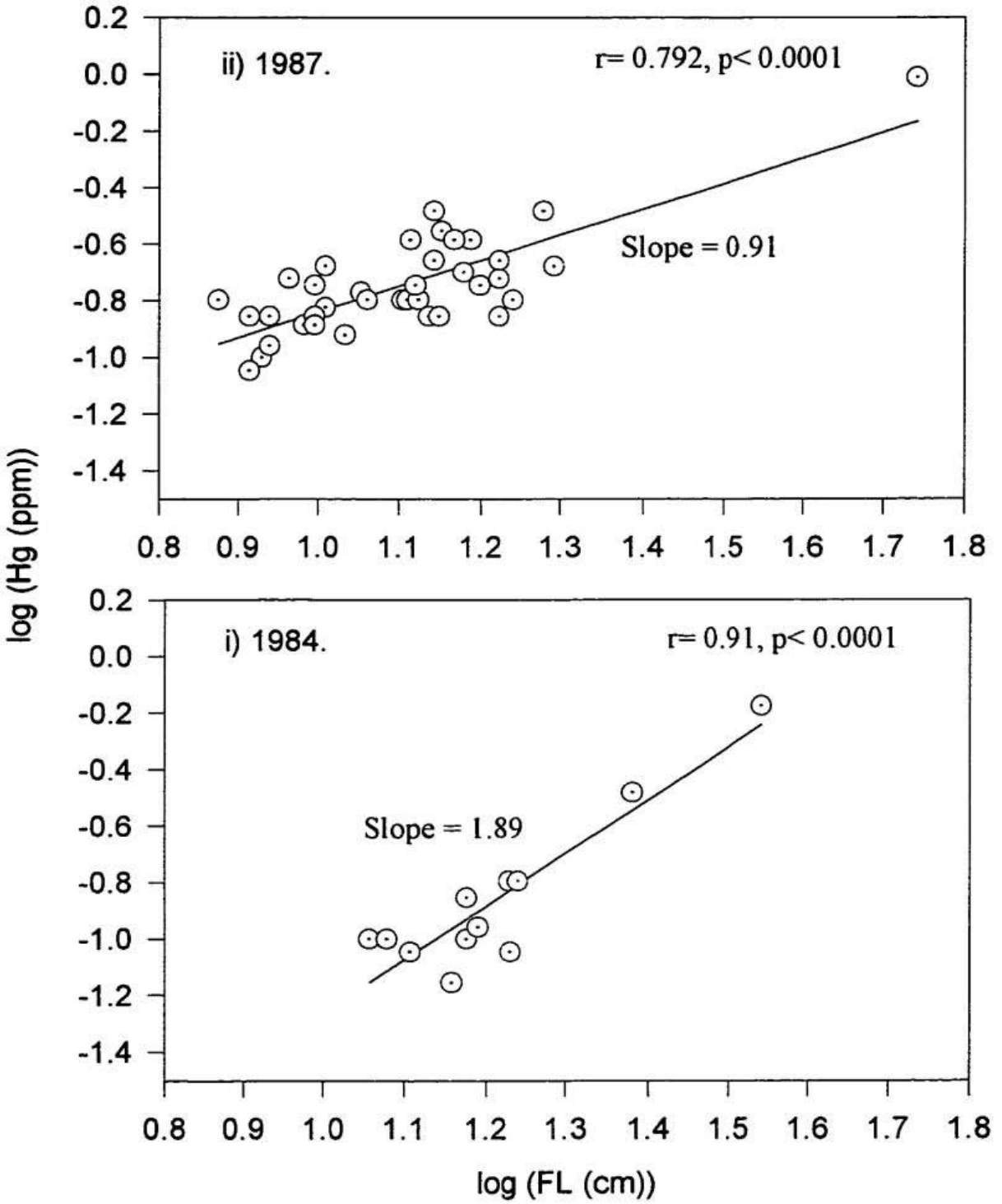


Figure A- 13.: Plots of log (Hg (ppm)) vs log (FL (cm)) for brook trout collected from control site, Rocky Pond for i) 1984 and ii) 1987. Solid lines represent the least square regressions.

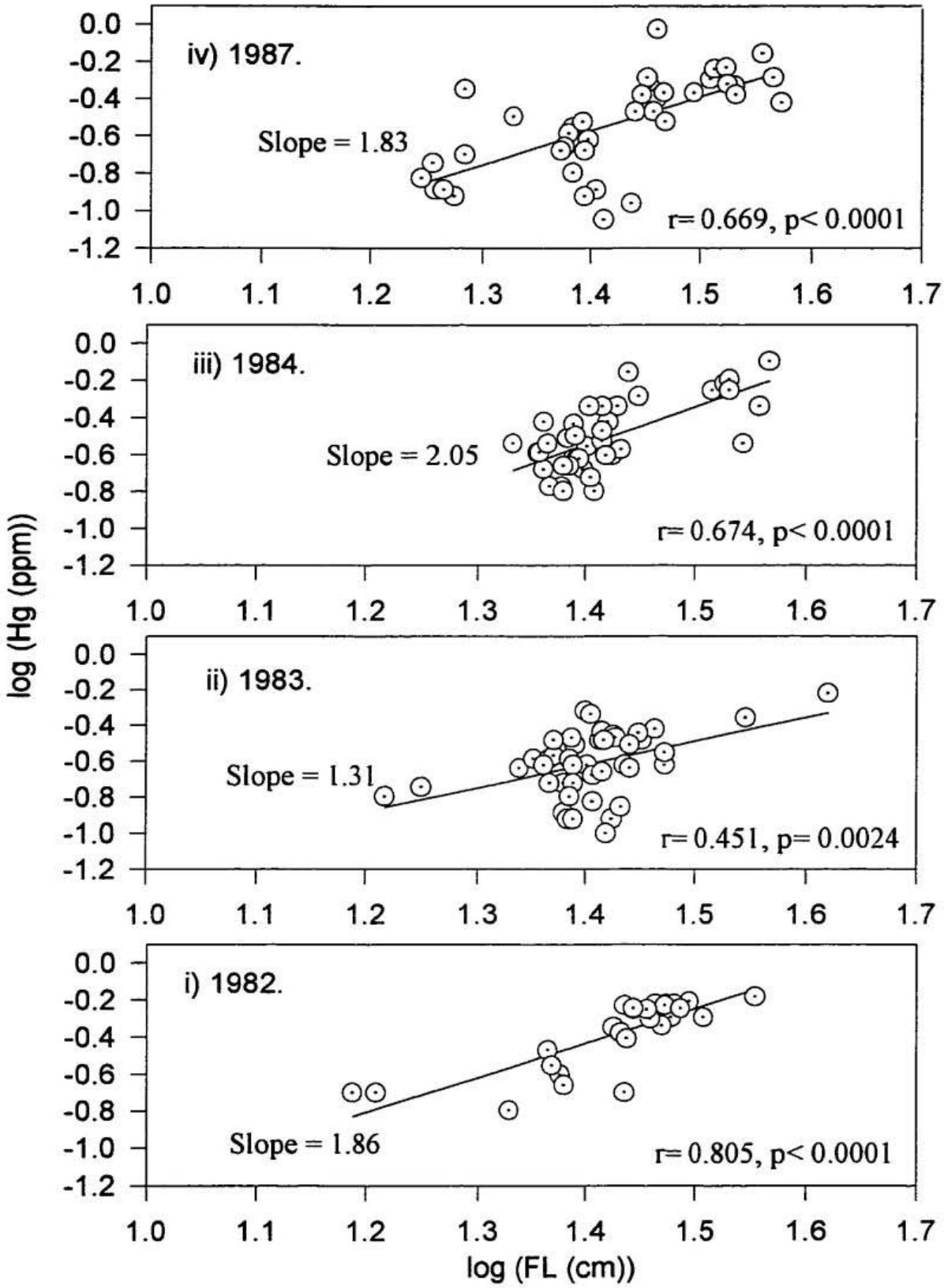


Figure A- 14.: Plots of log (Hg (ppm)) vs log (FL (cm)) for ouananiche collected from control site Rocky Pond for i) 1982, ii) 1983, iii) 1984, and iv) 1987. Solid lines represent the least squares regressions.

Appendix 2: Example of Exploratory Analysis

Analysis performed with Minitab Statistical Software (1992).

The following are the results from weighted regression analysis used to model the increase in mercury levels in brook trout across NF from reservoirs up to 5 years of age.

```
MTB > regress c2 (Hg) 1 c1 (Reservoir Age);
SUBC> weights c3;
SUBC> residuals c13;
SUBC> fits c14.
```

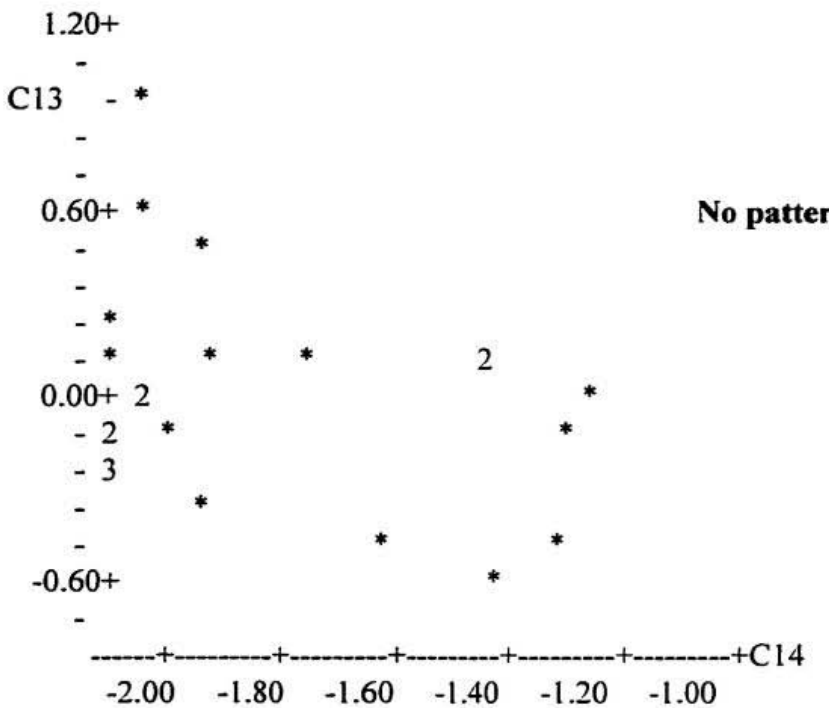
The regression equation is
upbthg = - 2.10 + 0.217 btupage

Predictor	Coef	Stdev	t-ratio	p
Constant	-2.09837	0.07666	-27.37	0.000
btupage	0.21703	0.03138	6.92	0.000

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	42.161	42.161	47.82	0.000
Error	21	18.513	0.882		
Total	22	60.674			

MTB > plot Residuals (C13) vs Fits (C14)



No pattern in residuals.

MTB > root Residuals

BIN	COUNT	RAWRS	DRRS	SUSPENDED ROOTOGRAM
1	0.0	-0.2	-0.39	. -- .
2	1.0	0.0	0.22	. ++ .
3	3.0	0.0	0.16	. + .
4	6.0	0.6	0.32	. ++ .
5	6.0	-0.3	-0.00	. - .
6	4.0	-0.5	-0.10	. - .
7	0.0	-2.0	-1.99	.---------- .
8	2.0	1.5	1.38	. +++++++ .
9	0.0	-0.1	-0.17	. - .
10	1.0	1.0	1.43	. ++++++++ .
11	0.0	-0.0	-0.00	. - .

No unusual observations.

IN DISPLAY, VALUE OF ONE CHARACTER IS .2 OO

MTB > hist Residuals

Histogram of C13 N = 23

Midpoint	Count	
-0.6	1	*
-0.4	3	***
-0.2	6	******
0.0	6	******
0.2	4	****
0.4	0	
0.6	2	**
0.8	0	
1.0	1	*

Histogram of residuals close to normal.

The plot of resuduals versus the fitted values, the rootgram of the residuals, and the histogram of the residuals all indicated normality; therefore the model was accpeted.

Appendix 3: Quality Assurance Test

Table C- 1. Results from quality assurance test.

Hg Content (ppm) February 1996	Hg Content (ppm) April 1996	Difference (ppm)[1]
1.01	1.00	0.01
1.81	1.79	0.03
0.94	1.08	0.14
1.35	1.46	0.11
1.21	1.35	0.14
0.74	0.81	0.07
0.87	0.98	0.11
0.82	0.86	0.04
1.09	1.10	0.01
0.91	1.06	0.15
1.05	1.12	0.07
0.97	1.02	0.05
0.85	0.82	0.03
1.05	1.05	0
Average Difference		0.069

[1]ANOVA ($\alpha = 0.05$) results indicated that there was no significant difference between Hg results from the February and April analyses ($F = 0.3404$; $p = 0.565$).